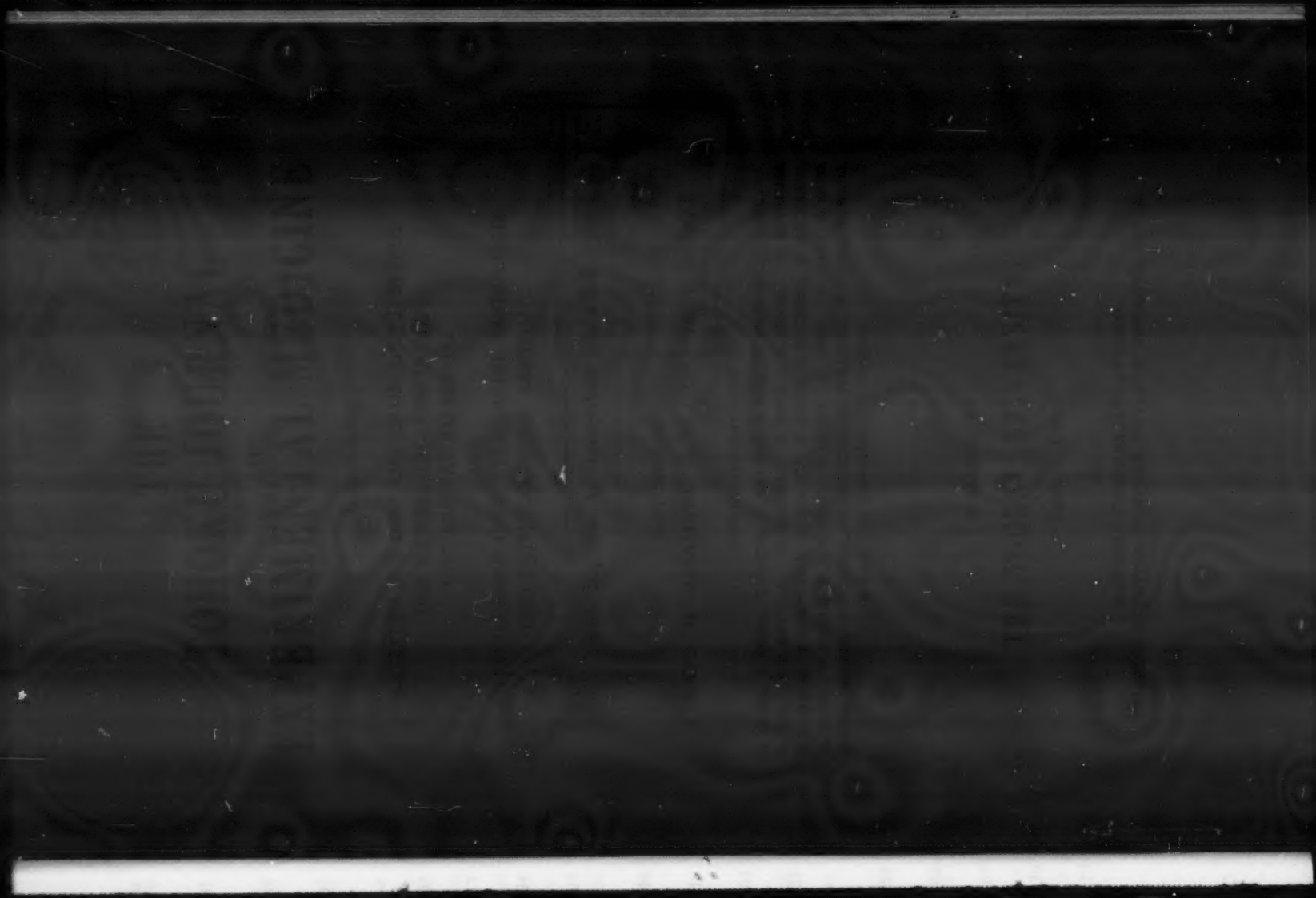

The Tohoku Journal of Experimental Medicine, Vol. 53, No. 1, 1951

Endemic Occurrence of Ariboflavinosis and Pellagra

Clinical Observations of so-called "Shibi" or "Gatchaki" in Tsugaru District

By

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(Received for publication, June 1, 1951)

Introduction

We have been quite interrested in a special syndrome which has been called under the dialectal name "Shibi" or "Gatchaki,"[1] and cases with such a syndrome are frequently found in the rural, (especially Tsugaru), district of Aomori Prefecture. The fact that such a special term —"Shibi" or "Gatchaki"—has been given to the syndrome in question by the inhabitants in this district, will suggest that this syndrome has been of endemic occurrence for a considerable long period in this area.

Philologically, "Shibi" means "roughness" and "Gatchaki" means probably "itching of anal and genital regions." Of course its significance seems to be different even in that district, so that some take it for an infectious or hereditary affection and others for a fatal disease, having seen cases developing cerebral symptoms. And it is said that "Shibi" attacks both young and old and will get worse in spring, and that in female, it will be initiated by pregnancy in a majority of cases. Basing upon clinical observations of the 100 cases accepted as of "Shibi" from among inhabitants in Tsugaru district, that were examined in our Hospital over a period from Feb. 1950 to Feb. 1951, we shall report the outline of this syndrome.

Clinical Observation

Chief complaints (Cf. Table I)

As will be seen from Table I, itching of anal and genital regions and headache were found in half the cases; uncomfortable feeling of the stomach, pain of the mouth and occular lesions such as poor or blurred vision, burning of eyes, lacrimation and roughness of eye lid skin—all

these combined—were found in about 30 cases; constipation in 15 cases,

TABLE I

Chief Complaints* of 100 Cases with "Shibi" or "Gatchaki"

Subjects	Incidence of complaints
Lassitude	52
Anal or genital itching	51
Headache	49
Uncomfortable feeling of stomach	31
Ocular lesions	31
Pain of mouth	28
Constipation	15
Burning of throat	8
Diarrhea	7
Pruritus of skin	6
Emaciation	5
Stiffness of shoulders	3
Ringing in ears	2
Cough	1
	300

* Only three main complaints were recorded from each patient with "Shibi" or "Gatchaki."

drowsiness or disturbed sleep in 11 cases, burning of the mouth in 8 cases, diarrhea in 7 cases, pruritus of the skin in 6 cases, emaciation in 5 cases, stiffness of the shoulders in 3 cases, ringing in the ears in 2 cases and cough in one case.

Local lesions (Cf. Table II)

Lips:—Cheilosis was found in 73 cases and angular stomatitis in 74 cases.

Tongue:—Lesions of the tongue were found in 91 cases, of which coated purplish tongue swollen due to chronic edema was found in 10 cases, smooth tongue in 16 cases, fissured tongue in 10 cases and magenta tongue in 27 cases.

Eyes:—In 74 cases, so-called "Shibi—eye" was found and this term includes various kinds and degrees of ocular lesion, such as conjunctivitis, pericorneal injection, vascularization and steamy opacity of the cornea and chronic blephalitis. It is not seldom that a considerable number of cases with trachoma may pass for cases of "Shibi-eye," but we found thickness and turbidity of palpebral conjunctiva in 7 cases which showed no evidence of trachoma.

TABLE II

Lesions of Skin and of Mucous Membrane in 100 Cases with "Shibi" or "Gatchaki"

Lesions	Cases
Glossitis	91
Angular stomatitis	75
Cheilosis	73
Angular blephalitis	52
Eczematous lesions of nasal orifice	35
Ocular lesions	74
Anal dermatose	46
Genital dermatose	52
Pigmentation of face	39
Dry dermatitis of neck	13
Itching of skin	15
Roughness and pigmentation of hand	70
Roughness and pigmentation of foot	42

Skin lesions:—Obstinate itching of the skin, universal or localized, in such regions as scrotum, neck and head, was complained of in 15 cases. Hyperkeratosis with hyperpigmentation—pellagrous skin—was found in 70 cases. Hyperpigmentation, almost ugly, of nipple was observed in 29% of the women with "Shibi." Petechial hemorrhage on the chest and on the upper arms was observed in 17 cases and its incidence seemed to be higher in summer than in winter.

Anal itching and genital itching are the most distressing complaints of "Shibi" and were recorded in 46 and 52 cases respectively. Respecting perianal lesions, fissures were found in 40% of the cases, edema in 30%, redness in 22% and ulceration in 8%. These anal lesions were more frequently met with in the cases with pellagrous symptoms than in those with ariboflavinotic symptoms alone. Fibrinous dermatitis and edema were constant findings of the vulva in females suffering from "Shibi," and with complaint of itching of genital regions.

Gastrointestinal lesions:—Hypoacidity and anacidity were found in 86 cases. Obstinate constipation was more frequently observed in the cases with ariboflavinotic symptoms than in those with pellagrous skin lesions, while the incidence of diarrhea showed inverse relation.

Burning of the throat, larynx or esophagus was complained of in 40 cases, in some of which dry cough or sensation of foreign body in esophagus accompanied those pharyngeal, laryngeal or esophageal burning.

Mental and nervous symptoms:—On the contrary to the cases with acute

pellagra, in those with ariboflavinotic signs alone none of mental and nervous symptoms were found. When one or more such ailments as lassitude, headache, ringing in the ears, myalgia, neuralgia, mental depression, sleeplessness or drowsiness had developed, " Shibi " is said to have become more serious or to have gone into advanced stage. Nystagmus, ptosis and ataxia were occasionally observed even in the cases without symptoms.

Pellagrous dementia was found in 8 cases out of 79 cases with typical pellagra admitted into our Hospital during the last 5 years.

Discussion

The greater part of Tsugaru district is paddy-rice-field, which will be covered with snow over a period from November to April, and a considerable part of the other area is mostly used for apple orchards. Lots left for the inhabitants of the district as the kitchen-garden for their own daily use are exceedingly limited, so the ratio of the kitchen-garden to the paddy-rice-field is 5:100.[1] The diet consists of mainly highly polished rice and of a small amount of green vegetables with a casual dish of fish or meat.

All the 100 cases with " Shibi " examined by us showed more or less the symptoms ascribed to vitamin B_2 complex deficiency, 84 cases of which showed none of detectable evidence of underlying diseases and the other were considered as cases of B_2-avitaminosis associated with tuberculosis (7 cases), syphilis (3 cases), hookworm infestation (2 cases) and starch injury or " Mehlnährschaden " of the Germans (4 cases).

In all the cases of " Shibi " pellagrous lesions of the skin were found to some extent, and in half of them itching of anal and genital regions was complained of. Itching of anal and genital regions has indeed been reported as a symptom associated with ariboflavinosis,[2] but when the expression " Gatchaki " is used in our region, it is understood that anal or genital itching is especially emphasized. Ts. Arakawa,[3] Professor of Pediatrics in the Hirosaki University, reported that macrocytic anemia was not uncommonly found in sick children in Hirosaki area of Tsugaru district and that these children should be considered as being in a state of " nutritional dystrophy." " Shibi " or " Gatchaki " in Tsugaru district may, as described above, be accepted as a syndrome which is characterized by pellagrous skin, ariboflavinotic signs and distressing itching of anal and genital regions and which may be ascribed to nutritional deficiency prevalent in the district.

Summary and Conclusions

1. The dialectal name " Shibi " or " Gatchaki " represents a syn-

drome which is very commonly found among the inhabitants in Tsugaru district of Aomori Prefecture.

2. "Shibi" or "Gatchaki" is a syndrome to be probably ascribed to nutritional deficiency and characterized by pellagrous skin lesions, ariboflavinotic signs and distressing itching of anal and genital regions.

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The Tohoku Journal of Experimental Medicine, Vol. 55, No. 1, 1951

The Most Typical Symptom of Infantile Beriberi, Preliminary Report

By

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(Received for publication November 16, 1951)

Beriberi was not an experimentally produced disease, but a real one found in man, so that a patient of beriberi may also present such symptoms as may have nothing whatever to do with thiamine deficiency. Human milk is a true secretion.[1] Nobody doubts that it is the only ideal food for a young infant. The exclusively breast-fed infant is, as far as nutrition is concerned, the happiest child throughout the world.

Now what is the most typical or unmistakable symptom of infantile beriberi? It is sudden death or cardiac death of a "healthy"[2] breast-fed infant. This symptom is, though it is only one symptom, the pathognomonic one of infantile beriberi. Without such fatality infantile beriberi would have been recognized as a clinical entity only much later.

What is the best treatment for fatal infantile beriberi? Even a massive dose of thiamine may fail to cure it. Then, what was the best treatment for such at the pre-vitamin age? Some cases could be cured, if they were weaned immediately and put even to "omoyu,[3] a very diluted gruel of polished or thiamine-free rice; because they had been transferred from poisonous milk to non-poisonous food, and because infantile beriberi is not so much a thiamine-deficient disease, as the result of acute poisoning (with a methyl glyoxal like substance) due to thiamine deficiency. Therefore, the essence of infantile beriberi ought to be sought in the pathogenesis of this sudden death of a "healthy" breast-fed infant.

Human milk is the only ideal food for a young infant throughout the world. The most typical symptom of infantile beriberi is *sudden cardiac death of a "healthy" breast-fed infant or "ideally" fed infant.*

References and Notes

1) Sato, A., Jikken Iho (Japanese), 1936. No. 244, 523. 2) Apparently healthy and well fed. 3) Of course, a long continued feeding on "omoyu" would then cause death due to inanition and multiple avitaminosis.

The Tohoku Journal of Experimental Medicine, Vol. 53, No. 1, 1951

On the Innervation of the Ventral Abdominal Wall in 10th Month Human Embryo

By

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(Received for publication, June 2, 1951)

Though the histological investigations on the innervation of the ventral abdominal wall have been carried out by Dogiel,[1] Timofejew,[2] Ramstrem,[3] and Cyon[4] etc., their results are no more useful, because they are all originated in the antiquated staining methods which had been used more than a half century before. Besides, not only their material was chiefly obtained from the animals, but also the object of the observation was limitted to the subserous tissue with peritoneum.

Then I took as the material the total abdominal wall from the pritoneum to the skin obtained from five human embryos of 10 months. After fixing the material in 10% neutral formol about one year, I made them in 40 μ sagittal and horizontal frozen sections and stained them by Seto's silver impregnation.[5] The microscopical examination has brought the following results.

Individual Views

1. Innervation of the subserous tissue, especially on the non-corpusclar endings

The nerve elements which distribute in the ventral abdominal wall are originated chiefly in nn. intercostales and composed of the medullated motor and sensory, and the non-medullated fine vegetative fibres. The latters anastomose in places on their course with the perivascular vegetative nerve elements. By the way, these intercostal nerves, reaching to the subserous tissue, pass into the nerve plexus extending plainly there. The thick or thin nerve bundles ramify from the plexus and divide into finer branches with the approach of peritoneum.

As the nn. interecostales lose their motor fibres on their course through the adominal muscles, they are in the subserous layer merely composed of both sensory and vegetative fibres. It is said by Dogiel[1] and Timofejew[2] that the medullated fibres sometimes divide into a few of branches at the

node of Ranvier, then anastomose with other medullated fibres. I could not meet such a view. On the contrary, nonmedullated fibres distribute to the periphery branching and anastomosing each other and finally pass into the terminalreticulum (Stöhr).[6)7)] As confirmed many rising authorities in various places of the body, this is the end-apparatus of the vegetative fibres, represented by the fine nerve-cord composed of neurofibrillar meshwork fulled with neuroplasm-mass containing Schwann's nuclei and does not end freely, but always anastomoses with the others to make as a whole a large network. This terminalreticulum developes considerably also in the subserous layer, and innervates the peritoneal epithelium, the vacular wall and the cells of the others' tissues chiefly by means of contact.

The sensory nerve fibres in the subserous layer pass into the noncorpusclar and the corpusclar terminations. The formers are far superior to the latters in quantity and seemed to be numerous owing to the long complicated course of the stem fibres, but not so dense virtually. They are sometimes represented by the most simple non-ramified sharp terminations (Fig. 1) just beneath the peritoneal epithelium, but mostly by the ramified endings (Fig. 2) with a few terminal branches. Dogiel[2)] and Timofejew[2)] saw the fine reticular bodies or saw-like plates at the tip of the terminal branches, but I could not meet such a view as well as the intraepithelial fibres entering the peritoneal epithelium.

Fig. 1

Fig. 2

Fig. 1. Non-ramified sensory nerve ending beneath the peritoneal epithelium of the upper region of the ventral abdominal wall in the 10th month human embryo. Horizontal section. Seto's silver imprenation, X 550, reduced to $\frac{1}{2}$.

Fig. 2. Simply remified sensory nerve ending beneath the peritoneal epithelium of the middle region of the ventral abdominal wall in the 10th month human embryo. Sagittal section. Same statining, X 750, reduced to $\frac{1}{2}$.

2. Corpusclar terminations in the ventral abdominal wall

They are divided into the Pacinian bodies and the special ones re-

Fig. 3

Fig. 4

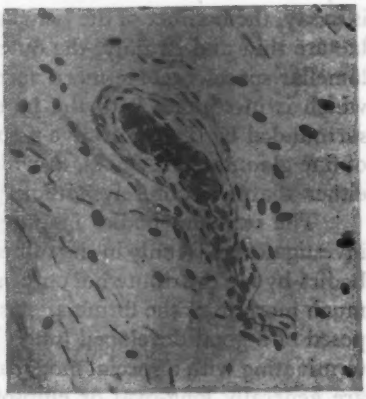

Fig. 3. A Pacinian body found in the subserous tissue of the middle region of the ventral abdominal wall in the 10th month human embryo. Horizontal section. Same staining, X 450, reduced to 2/5.

Fig. 4. A typical special end-corpuscle seen in the teninous inscription of m. rectus abd. in the 10th month human embryo. Transversal section. Same staining, X 550, reduced to 1/3.

Fig. 5

Fig. 6

Fig. 5. The twin formation of the special end-corpuscle found in the ventral plate of vagina musculi recti abd. in the 10th month human embryo. Transversal section. Same staining, X 450, reduced to 1/3.

Fig. 6. The special end-corpuscle found in the perimysium ext. of m. obliq. ext. abd. in the 10th month human embryo. The nerve fibre in the inner bulb divides into 3 terminal branches. Horizontal section. Same staining, X 450, reduced to 1/3.

cently discovered by me. The formers are found rarely and only in the subserous tissue, but develope far better than those in the palm (Wada)[8] and the clitoris (Yamada)[9] in the embryos of the same months, so that

they are already in this fetal life brought to the rough completion. That is to say, the number of the lamellae is more than 20, and the inner lamellae are thin and strainly, the outer ones thick and wave-like. The interlamellar spaces become more narrow with the approach of the inner side which is in contact with the inner bulb, and of the outer side which is surrounded by the connective tissue capsule. The inner bulb is composed of fine glanular substances, in which the entered nerve elements terminate either sharp or obtusely without showing so much ramification (Fig. 3).

The terminal bodies in the abdominal wall ever reported by some investigators exist only in the subserous tissue. On the contrary, the special bodies by me distribute not only in this tissue, but also in other tissues even much more than the formers. As seen in Fig. 4, 5, 6 and 7, they are composed of a good developed inner bulb, which contains a thick nerve fibre terminating with a special mode, and a few lamellae surrounding it. They are generally long-oval or ellipsoid in form, 35–200 μ in length and 30-60 μ in width, namely far smaller than the Pacinian bodies. The distal pole is obtuse, the proximal pole which the nerve fibre penetrates is more or less sharp. Furthermore, it is noticeable that there is seen the formation of a characteristic cylinder-body, termed by me the vestibulum corpusculi, before the proper body. In short, my special body is represented as a special corpuscle having a peculiar vestibulum. But it often divides itself into two bodies, to show a twin-like formation, but the vestibulum is always common to both of them (Figs. 5 and 7).

Writing more in detail on my special body, its thin connective tissue lamellae arrange generally in 2–5 layers, containing here and there flat spindly unclei rich in chromatin. Along the surface of the lamellae are seen very fine fibrills resembling to the lattice-fibres which show the fibrous connection between each of the lamellae through the interlamellar spaces. The lamellae in the proximal pole of the body disappear with passing into the connective tissue capsule surrounding the vestibulum corpusculi.

The inner bulb is so large, that it occupies the half or sometimes nearly the whole part of the body, and consists of many special nuclei and somewhat granular and dark-reddish stainable substances. The special nuclei are flat and round or oval, poor in chromatin, and resembling nearly to the special nuclei lieing in the central nuclei-gathering of the muscle-spindle as described later. They distribute in general equally in the inner bulb, having but a tendency to somewhat dense arrangement along the terminal part of the nerve fibre. The cell-boundary concerning these nuclei is not clear, so that the inner bulb shows a kind of cyncytium, which should be compared with the cell-plasmodium in the surroundings of the sympathetic nerve cells (Nebenzellen-plasmodium by Stöhr)[7] and of great significance not only structurally, but also functionally. Then, I

Fig. 7

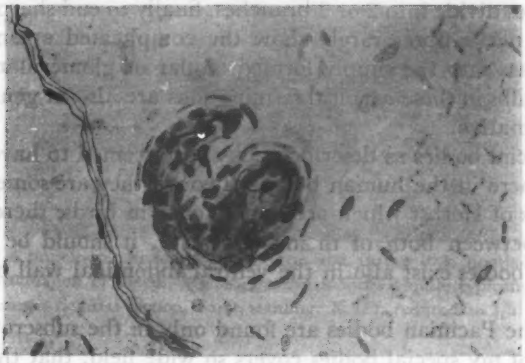

Fig. 7. A twin-like special end-body found in the perimysium ext. of m. obl. ext. in the 10th month human embryo. The nerve fibre in the inner bulb on the right ramifies in some branches which end sharply after the winding course. Horizontal section. Same staining, X 700, reduced to ½.

suppose, as many rising authorities, that this cell-plasmodium will be of endocrinous nature like the Kohn's accessory cells, and the stimuli to the corpuscle cause primarily the exitement of the cell-plasmodium, namely the secretion of special endocretes, which secondly stimulate the terminal nerve fibres, finally to transmit the excitement to the center.

The vestibulum corpusculi including a nerve fibre is composed of proper substance and connective tissue capsule, generally cylindrical in form and a little thinner than the inner bulb and decreases its thickness with the approach of the proximal pole of the corpuscle, to send the nerve fibre into the inner bulb from its point. The proper substance is hard stainable and contains a few of special nuclei of syncytial nature, so that it seems to belong to the peripheral neuroglia like the inner bulb. Its proximal pole seems to pass into the medulaary sheath of the stem fibre without any boundary. The connective capsule continues at the distal pole to the lamellae of the body and at the proximal pole to the Henle's sheath.

One of the medullated fibres separated from a small bundle represents the change of thickness not so much after penetrating the vestibulum and takes a characteristic snaky or wavy course in it. The nerve fibre to the single corpuscle is not ramified, but that to the twin bodies divides into two branches. Penetrating the inner bulb of the corpuscle, the nerve fibre takes in general suddenly the straight course and shows gradually the neurofibrillar enlargement, to end finally with a clubbed termination. In addition to this typical terminal mode, I could recognize not so rarely the atypical terminal formations (Figs. 6 and 7), where the nerve fibre is

characterized with the change of thickness and the peculiar winding course, and sometimes divide into 2 or 3 branches, finally to end sharp or obtusely. Besides, the nerve fibres rarely show the complicated arrangement and sometimes pass into the simply formed anular or glomerular end-bodies. The inner bulbs in these atypical terminations are also in general more or less transformative.

The special bodies as described above are seemed to have never been found anywhere in the human body. Though they are somewhat similar to the bodies of Herbst which are said to exist in birds, there are distinct differences between both of them. However, it should be confirmed if these special bodies exist also in the ventral abdominal wall of the human adult.

While the Pacinian bodies are found only in the subserous tissue, the localisation of my special bodies covers so wide field, that they distribute not only in it, but also in other tissues. The order of the locals arranged by the discovering frequencies of the special bodies is as follows: 1. inscriptiones tendinae of m. rectus abd. and linea alba, 2. ventral plate of vagina m. recti abd. and its surroundings, 3. dorsal plate of vagina m. recti abd. and its surroundings, 4. lateral border of vagina m. recti abd., 5. subserous tissue under m. rect. abd., 6. fascia m. obliqui ext. abd., 7. fascia transversalis and its subserous tissue and 8. intramuscular connective tissue in the periphery of m. rect. abd.

As described above, the special bodies exist mostly in m. rectus abd. and decrease gradually with the approach of the lateral abdominal muscles. Most frequently they are seen within or in contact with the tendon tissue or in the extrernal perimysium. Thus, it might be thought that their special localisation will depend upon the importance of the physiological significance.

3. Muscle-spindles in the abdominal muscles

Dogiel[10] found the muscle-spindles in mm. rectus et transversus abd. of animals, though he could not obtain the satisfactory results, because he stained the materials with the methylenblue. Now, it seems to be very I who studied on this subject in detail taking the materials in the human and staining with the brilliant silver method.

The muscle-spindles in the abdominal muscles in 10th month human embryo (Figs. 8, 9 and 10) are composed of a few fine striated muscle fibres i. e. Weismann's fibres and a thick connective tissue capsule surrounding them, as like as those in other striated muscles, and found in every abdominal muscle, but in m. rect. abd. most numerous and well developed. In comparison with the muscle-spindles in the tongue (Nakayama)[11] and thenar muscles (Kura)[12] of the same month embryo, my abdominal muscle-

Fig. 8

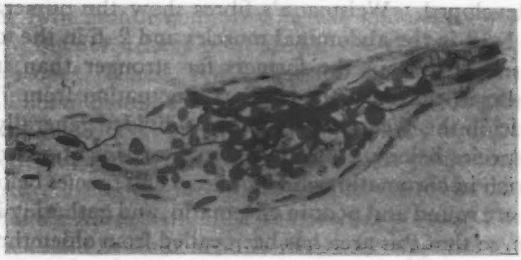

Fig. 8. A muscle-spindle found in m. rectus abd. in the 10th month human embryo. The chief termination of the thick sensory nerve is formed quite typically in the central nuclei gathering. Sagittal section, Same staining, X 525, reduced to 2/5.

Fig. 9

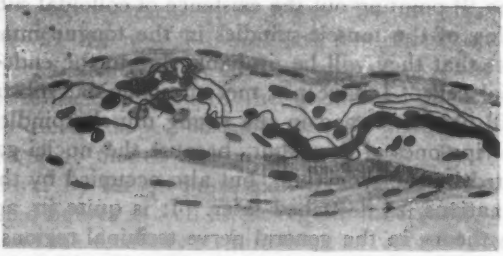

Fig. 9. A muscle-spindle found in m. transv. abd. in the 10th month human embryo. The special winding course of the terminal branches and a plate-like terminal end-body are seen clearly in the central nuclei gathering. s sensory stem-fibre. Horizontal section. Same staining, X 840, reduced to 2/5.

Fig. 10

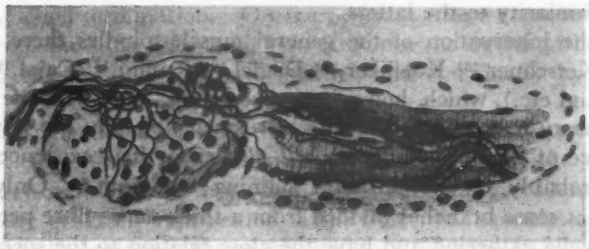

Fig. 10. A muscle-spindle in m. obl. int. abd. in the 10th month human embryo. Many terminal branches of the chief termination which end with each various terminal body are seen on the left, the accessory termination is seen on the right. Horizontal section. Same staining, X 500, reduced to 3/5.

spindles are much more than the formers, may be several times in number and better developed. Weismann's fibres show the numeral fluctuation remarkably, 1–12 in the abdominal muscles and 2–6 in the tongue muscles (Nakayama). So, it is in the formers far stronger than in the latters. They show also in thickness the similary fluctuation from 7 to 30 μ.

The nuclei in the Weismann's fibres are divided generally into 2 kinds. To the first species belong the proper nuclei of this fibres which are oval in form and rich in chromatin, while to the second species belong the special nuclei which are round and poor in chromatin, and gathering in the swelling of the spindle, so that this area has been called from olden times the central nuclei gathering (Felix,[13] Baum,[14] Amerbach[15]). So, the swelling formation may be caused by their gathering. Accordingly, the connective tissue capsule becomes very thin in this place and Weismann's fibres also become so degenerative, that they come in places to disappear incontinously.

Nakayama,[11] pointing out the existence of colloidal substances in the nuclei gathering of the muscle-spindles in the tongue muscle of human adult, supposes that they will be probably due to the endocrine function of these special cells. I have not met the colloidal substances. But, I think they will not be formed in the young muscle-spindles, but able to appear in the ripe ones of the adult, because the nuclei gathering is not only represented by the syncytium, but also occupied by the complicated sensory terminations, as described later. It is quite in agreement with the endocrine theory in the general nerve terminal regions advocated by many rising authorities and easily imaginable, that this area will also have a endocrine function reacting to the external stimuli.

By Nakayama[11] the special nuclei seem to be represented by the differenciation of the proper muscle nuclei. But, I suppose they might grow from the Schwann's nuclei belonging to the sensory nerve fibres, like the special nuclei in the inner bulb of the terminal bodies, because they are in great similarity to the latters.

On the innervation of the general muscle-spindles there are many reports (Kerschner,[16] Koelliker,[17] Dogiel,[10] Ruffini,[18] Cajal,[19] Tello,[20] Boeke[21] and etc.), which, however, are concerned only with the ramifying of the sensory nerve fibres, and do not touch not only the physiological significance of the special nuclei, but also even their existence. This is caused probably to the unskilful staining techniques. Only Inoue[22] reports that some branches divided from a thick nerve fibre penetrate the central nuclei gathering and have the close relation to the special nuclei, though he showed no figures in his description. On the contrary, Nakayama[11] states very plainly his detailed views on this subject.

My observations on the innervation of the muscle-spindles do not differ

from those of Nakayama so much, but only that the development of their innervation in 10th month embryo is in the abdomen far stronger than in the tongue. Besides, I have been able to contribute some of new views to Nakayama's ones. Then, I will describe them as follows.

The small nerve bundle for the muscle-spindle is composed of sensory, motor, and non-medullated fine fibres. The sensory and the motor fibres are medullated, but the formers are 5–6 or even more times as thick as the latters. After losing the medullary sheath, the motor fibres terminate in the Weismann's fibres with their motor end plates. The non-medullary fibres were found by some of authorities and supposed to be sympathetic. Nakayama[11] says, however, they also would be sensory, as their terminal apparatus seem to end freely. In my views, they are probably belonged to the vegetative fibres, because they show the fine neurofibrillar net-formation in places on their course (Fig. 10).

It is not too much to say that the nerve fibres for the muscle-spindle are almost occupied by the sensory fibres, because the motor and the vegetative fibres for the muscle-spindle are far inferior to the formers especially in the complexity of the nerve termination. The sensory terminations in the muscle-spindles of the abdominal muscles in the 10th month embryo are developed far more strongly than those of the tongue muscles in the same month embryo (Nakayama). By the high magnification, moreover by the oil immersion these terminations represent the very complicated figures as well as the regular innervation and are divided into the chief and the accessory terminations, as reported already by Nakayama.

A thick medullated fibre for the muscle-spindle loses its medullary sheath as soon as it penetrates the connective tissue capsule and divides into several branches, the most of which advance to the nuclei gathering, to ramify furthermore in many terminal branches. Thus, the sensory termination for the muscle spindle is greatly formed in the nuclei gathering. This is the chief termination. On the contrary, there are a few branches divided from the stem fibre, which do not enter the nuclei gatheiring, but run along the Weismann's fibres forward and end finally by encircling them. This is the accessory termination.

Observing in detail the chief termination, all the sensory nerve branches to the nuclei gathering run with their subbranches along the sarcolemm either spiral or looplikely, but often quite irregularly. These terminal branches are generally represented as fine fibres, but sometimes they show the neurofibrillar enlargement or the change of thickness in places on their course and terminate with each peculiar end-apparatus in the outside of the sarcolemm i. e. epilemmally, or going into the sarcoplasm i.e. hypolemmally. There are some of them which terminate along the Weis-

mann's fibres in this area.

The end-apparatus of the terminal branches are quite various. In the first place are shown the sharp endings (Figs. 8 and 10), in which the tip of the terminal fibres is formed sharply. They seem to be great in number especially in the growing muscle-spindles. The second are the knob-like endings, which are represented by the nervous swellings in the tips of the terminal branches. They are various in size as well as in form (Figs. 8 and 10). The third endings are the glomerular apparatus found rarely. In the fourth place come the plate-formed endings, in which the tip of the terminal branches is characteristic with the plate-like neurofibrillar enlargement. They often show triangle bodies, which can send out fine fibres from every apex (Figs. 8 and 10). It is noticeable, that all the terminal endings above described frequently terminate in contact with the special cell nuclei.

Next comes the accessory termination. This is far more simple than the chief termination in structure and composed of a few branches divided from the stem fibre. Here is not found such a hypolemmal terminal mode as seen in the chief termination. But, only a few of the special nuclei resembling to the Schwann's nuclei which accompany the nerve fibres are recognized evidently. Then, such a mechanism of impulses transmission as in the chief ternination should come into existence also in the accessory termination. But, this accessory termination is far more inferior to the chief termination in scale, so that this mechanism of the sensory reception in the muscle-spindle would be done probably in the most in the cheief termination.

4. Innervation of the skin in the ventral abdominal wall

It seems no histological investigation on this subject has been done, as far as I know. The nerves for this skin are represented by the nerve bundles of various thickness, which consist of medullated sensory and non-medullated fine vegetative fibres. Penetrating the subcutaneous tissue, they come into the sweat glands layer and make the plainly extending nerve plexus with their branches. Here is the extending of the vegetative fibres especially enormous and a number of fine fibres finally develop to the terminalreticulum, which surrounds the sweat glands, penetrates successively their proper membrane and the smooth muscle fibres and runs along the basal part of the gland cells, however, without entering them. Besides, these vegetative fibres anastomose on their course with the perivascular plexus.

Many nerve bundles from the plexus in the sweat glands layer penetrate the reticular layer of corium accompanying the blood vessels and reach the papillar layer. The majority of them proceed to the basis of the

follicle glands, to run further into the follicle neck between the follicle gland and the external follicle sheath. The vegetative elements in them distribute widely forming the terminalreticulum in the connective tissue follicle, m. arrector pili and the connective tissue of the papillar layer (Fig. 11).

The sensory fibres terminate mostly as so-called hair nerve fibres in the special place called the hair-neuroshield or neurotube (described later) like in other skin with hairs, for example head-skin (Seto),[23] pars cutanea of the lip (Seto etc.),[25] while a very few of them advance in the papillar layer and terminate near the epidermis with the simple endings. This fact means that the sensibility of the skin with hairs will be received almost by the hair nerves.

The sensory termination beneath the epidermis in the 10th month embryo is made by means of that the medullary fibres lose their medullary sheath, as they reach the terminal region, take the loop-shaped course and terminate sharply, almost without branching (Fig. 12). I suppose that this terminal mode will not vary so much even in the adult, because also in the head-skin and the pars cutanea of the eye-lid[24] the terminations under the epidermis are never complexly constructed.

It is very interesting that the terminations of the sensory hair nerve fibres in the ventral abdominal wall of the 10th month embryo are developed so remarkably (Figs. 13, 14 and 15), that there are seen a number of such terminations as being about no inferior to those in the head-skin and the eyelid in the adult. Their terminal mode consists in, as shown evidently also in some of works of our laboratory, that the nerve elements terminate in the gathering part of the circular arranged special cells, which is called by Seto the hair-neuroshield when it is formed like a shield at one side and the hair-neurotube when formed like a tube surrounding the outer follicle sheath. The terminal formations are divided into following several types i.e. the plexus-formed, the arborized, the zig-zag and the atypical terminations.

Fig. 13 shows a plexus-formed termination, in which the several sensory fibres divide into 2–5 subbranches after entering the special cells zone, which represent every neurofibrillar swelling in places on their characteristic winding course and finally terminate sharply in the upper part of this zone. Fig. 14 should be probably belonged to the young form of a zig-zag termination in the adult, because there are seen some zig-azg fibres among the plexus-like arranged terminal fibres. Fig. 15 shows a arborized termination where many terminal branches divided from a thick stem fibre distribute like the tree. By the way, it is common to all the terminations, that the terminal nerve elements relate very densely with the special cells often with their nuclei in this special cells zone. Besides, it seems that

Fig. 11

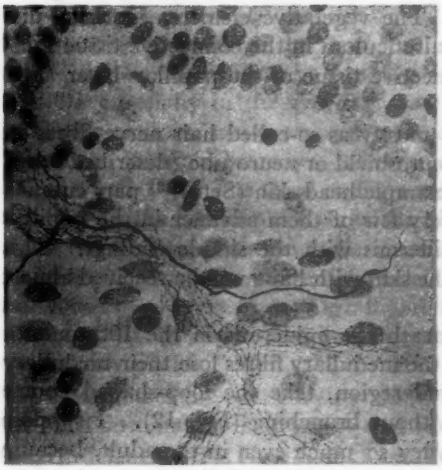

Fig. 12

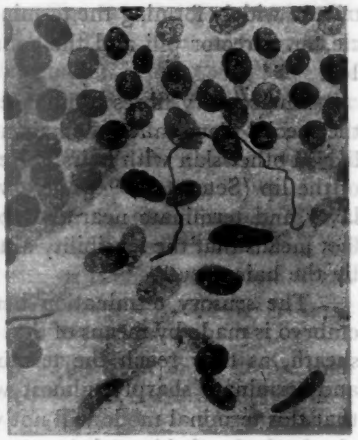

Fig. 11. A non-ramified sensory nerve ending (s) and the vegetative terminalreticulum (t) found in the stratum papillare of the abdominal skin in the 10th month human embryo. Horizontal section. Same staining, X 840, reduced to 2/5.

Fig. 12. A non-ramified sensory nerve ending running with the special winding beneath the stratum papillare of the abdominal skin in the 10th month embryo. Sagittal section, Same staining, X 840, reduced to 2/3.

Fig. 13

Fig. 13. The plexus-formed sensory end-apparatus found in the neuroshield (Seto) of the hair-follicle in the ventral abdominal skin. 10th month human embryo. Sagittal section. Same staining, X 840, reduced to ½.

Fig. 14

Fig. 15

Fig. 14. A young form of the zig-zag termination found in the neuroshield (Seto) of the hair-follicle in the ventral abdominal skin. 10th month human embryo. Sagittal section. Same staining, X 840, reduced to $\frac{1}{2}$.

Fig. 15. An arborized termination found in the neuro-shield (Seto) of the hair-follicle in the abdominal wall. 10th month human embryo. Horizontal section. Same staining, X 700, reduced to 2/3.

here in the abdominal skin exists almost no difference between the neurroshields and the neurotubes in number. Then, it is supposed that the adominal skin should have the stronger sensibility than the head-skin where the neurotubes are far inferior to the neurotubes, though the hairs are composed of typical coarse hairs.

Summary

In the ventral abdominal wall of the 10th month human embryo,the sensory nerve fibres in the subserous layer pass into the non-corpusclar and the corpusclar terminations. The formers are far superior to the latters in quantity and divided into the non-ramified and the ramified endings. The latters are likely divided into the Pacinian bodies and the special ones discovered by me. The Pacinian bodies are found rarely and only in the subserous tissue, but develope almost to the rough completion.

The special bodies distribute not only in the subserous tissue, but also in other tissues, even much more than the Pacinian bodies, are composed of a good developed inner lulb and a few lamellae surrounding it, and often divide itself into two bodies, to show a twin-like formation. Furthermore there is seen as a appendage of this body the formation of a cylinder-body termed the vestibulum corpusculi.

The inner bulb occupies the half or sometimes nearly the whole part of the body, and consists of many special nuclei and granular and dark-

reddish stainable substances, showing no cell-border like a syncytium. The vestibulum is composed of the proper substance which contains a few of special nuclei of syncytial nature and the connective tissue capsule. A medullated fibre penetrates the vestivulum after losing its myelin and takes a wavy course in it. Reaching the inner bulb of the proper body, the nerve fibre takes in general suddenly the straight course and end finally with a clubbed termination. But some of the nerve fibres show the change of thickness and the peculiar winding course, and often divide into some branches.

The muscle-spindles in the abdominal muscles in the 01th month human embryo are composed of a few Weismann's fibres and a thick connective tissue capsule like those in other striated muscles, and found in m. rectus abd. most numerous and well developed. In comparison with the muscle-spindles in the tongue and the thenar of the same month embryo, the abdominal muscle-spindles may be several times in number and much better developed.

The nuclei in the Weismann's fibres are divided into the proper nuclei and the special nuclei. The formers are oval and rich in chromatin, while the latters are round and poor in chromatin, and gather in the swelling of the spindle (central nuclei gathering by Felix and etc.). So the swelling formation may be caused by their gathering. Accordingly, the connective tissue capsule becomes very thin in this place and Weismann's fibres also become so degenerative, that they come in places to disappear incontinously. It seems that the special nuclei might grow from the Schwann's nuclei belonging to the sensory nerve fibres, like the special nuclei in the inner bulb of the terminal body.

It is not too much to say that the nerve fibres for the muscle-spindle are almost occupied by the sensory fibres, because the motor and the vegetative fibres are far inferior to the formers. The sensory terminations show the very complicated figures as well as the regular innervation and are divided into the chief and the accessory terminations. A thick medullated fibre for the muscle-spindle loses its medullary sheath and divides into several branches, most of which advance to the nuclei gathering, to ramify furthermore. This is the chief termination. On the contrary, there are a few branches which do not enter the nuclei gathering, but run along the Weismann's fibres forward and terminate finally by encircling them. This is the accessory termination, which is far inferior to the chief termination in scale.

In the cheif termination, the nerve branches to the nuclei gathering run with their subbranches along the sarcolemm spirally or looplikely, or often quite irregularly and terminate with each peculiar end-apparatus either epilemmally or hypolemmally. The terminal endings sometimes

remarkably terminate in contact with the special cell nuclei.

The sensory fibres in the skin terminate mostly as so-called hair nerve fibres in the special place called the hair-neuroshield or neurotube (Seto) like in other skin with hairs, while a very few of them advance in the papillar layer and terminate near the epidermis with the simple endings.

The terminations of the sensory hair nerve fibres in the abdominal wall are developed so remarkably, that there is seen a number of such terminations as being about no inferior to those in the head-skin and the eyelid in the adult (Seto). The terminal formations are divided into the plexus-formed, the arborized, the zig-zag and the atypical terminations, though they are not yet enough completed. It seems that here in the abdominal skin exists almost no difference between the neuroshields and the neurotubes in number.

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The Tohoku Journal of Experimental Medicine, Vol. 55, No. 1, 1952

Beriberi and Pyruvic Acid

By

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(Received for publication, November 16, 1951)

More than 100 papers[1] have been published from our Clinic and Laboratory since 1930 concerning beriberi esp. thiamine deficiency of lactants and infants. Those who have seen these papers must have wondered whether we were even aware of the world athiaminosis literature which had been centering on pyruvic acid, while our treatise must seem to be centering upon methyl gloyxal——a metabolite which modern theory of avitaminosis has abandoned almost utterly, so that if recent literature (books)[2] on athiaminosis does ever mention methygloyoxal, it is almost from our sources.

The only reference from our Laboratory that ever treated pyruvic acid is Ts. Arakawa's[3] treatise. Even then, I stated at the end of his treatise to the effect that this colorimetric result ought to be checked by strict chemical determination.

Why this reservation? Why such an apparent defiance at the current pyruvate theory as above mentioned?

Soon after our human milk study was started, we began to have a firm belief in "methyl glyoxal" as beriberi poison[4](1934). Of course we had not ignored the world wide opinion of pyruvate as the poison, and we made preliminary animal experimentaion in the same way as in the case of glyoxals. But such a striking deleterious effect upon animals' health was not observed. So all the rabbits used were killed, but their hearts were not found enlarged either. Later, Wako[6] in our Laboratory tried a similar experiment with freshly prepared pyruvic acid and came to the same conclusion.

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The Tohoku Journal of Experimental Medicine, Vol. 53, No. 1, 1951

Biochemical Studies on Carbohydrates

CXLVI. Mucosin

Third Report

By

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(Received for publication, June 10, 1951)

The enzyme complex from snail liver contains β-glucuronidase but not α-glucuronidase.[1] On incubation with it of desaminomucosin increase of iodine use occurred, and of the split products glucuronic acid could be separated as cinchonidine salt. Therefore, mucosin is β-glucuronide.

Experimental

Preparation of Free Desaminomucosin and Its Sodium Salt

Although desaminomucosin crystallizes well as cinchonidine salt,[2] this salt is not immediately applicable to the enzymic study below, because cinchonidine interferes with giving a sharp end point in thiosulfate titration of excess iodine. Hence free desaminomucosin and its sodium salt were prepared for that purpose. The yield of the former substance was small. The procedures were as follow.

a) *Free desaminomucosin.* 300 mg. of desaminomucosin cinchonidine salt were taken up in 20 cc. of water, the saturated $Ba(OH)_2$ added up to a pH of 9.0 and after standing overnight in an ice chest, the deposits were filtered off and well washed with cold water. The filtrate together with the washing was nearly freed from barium with H_2SO_4 (1 *N* and 0.1 *N* solution), distilled in vacuo almost to dryness, then again diluted with 5 cc. of water and filtered. The filtrate here was condensed to about 1 cc., followed by precipitation with 20 cc. of abs. alcohol and the precipitates were centrifuged, washed with abs. alcohol and dried. 42 mg. of a white amorphous powder were yielded. It was readily soluble in water. Molisch and Elson-Morgan, negative; Goldschmiedt and Neuberg-Saneyoshi, positive. Hot Fehling solution was reduced.

Analysis:
Ash ($BaCO_3$).

2.052 mg. substance, 0.029 mg. ash (1.41%).
Barium is calculated at 0.98%.
Iodine use as glucose (Macleod and Robison).
4.73 mg. substance, 1.397 cc. 0.02 *N* iodine used.
94.21% $C_{12}H_{18}O_{11}$ + 5.79% $C_{12}H_{17}O_{11}\frac{1}{2}Ba$. Calc. 52.6%, found 53.1%.
Glucuronic acid.
Indole method: 0.81 mg. substance (in 1 cc.), 0.464 mg. glucuronic acid (57.3%).
Oricinol method: 0.53 mg. substance (in 1 cc.), 0.303 mg. glucuronic acid (57.2%).
94.21% $C_{12}H_{18}O_{11}$ + 5.79% $C_{12}H_{17}O_{11}\frac{1}{2}Ba$. Calc. 56.7%.
Optical rotation in water.

$$[\alpha]_D^{18} = \frac{-0.07 \times 100}{1.29 \times 1} = -5.43°.$$

Mutarotation did not occur.

b) *Desaminomucosin sodium salt.* 500 mg. of the cinchonidine salt were dissolved in 20 cc. of water and after addition of 1 *N* NaOH up to pH 9.0, stood overnight at room temperature (9°C). The precipitates were separated off on a funnel and washed with water. And the filtrate and washing were distilled together in vacuo. The nearly dry residue was perfectly liberated from the insoluble part by taking up in 10 cc. of water and filtration. Again the filtrate was distilled and the remaining syrup was dehydrated and washed with abs. alcohol. The amorphous substance obtained was reprecipitated from 1 cc. of water by the aid of abs. alcohol and after washing similar to above, placed in a desiccator. 210 mg. of a greyish powder were yielded. The product was water-soluble and gave the same sequels in qualitative assays as the free acid.

Analysis:
N (Micro Kjeldahl).
4.03 mg. substance, no ammonia produced.
Iodine use as glucose (Macleod and Robison).
5.97 mg. substance, 1.605 cc. 0.02 *N* iodine used.
$C_{11}H_{16}O_{10}(CHO)Na$ (360). Calc. 50.0%, found 48.6%.
Glucuronic acid.
Indole method: 0.78 mg. substance (in 1 cc.), 0.423 mg. glucuronic acid (54.4%).
Orcinol method: 0.51 mg. substance (in 1 cc.), 0.276 mg. glucuronic acid (54.1%).
$C_{12}H_{17}O_{11}Na$. Calc. 53.9%.
Ash (Na_2CO_3).
10.21 mg. substance, 1.54 mg. ash.
$C_{11}H_{17}O_9COONa$. Calc. 14.7%, found 15.1%.

Digestion of Desaminomucosin by β-Glucuronidase of Snail Liver

The procedure which Nagaoka applied in the study of galactosidases[3] was employed. The snail enzyme solution was prepared also according to him,[4] but no decolorization with charcoal was conducted (pH of the enzyme solution, 4.5). The substrate-buffer solutions contained 0.025

mole sodium salt of desaminomucosin per cc.

To determine the optimum pH for the cleavage were incubated the mixtures which were composed of 1 cc. of a substrate-buffer solution and 0.3 cc. of the enzyme solution (1 drop of toluene was added for avoiding putrefaction) for 2 hours. Table I shows the increases of iodine use by 0.5 cc. of the mixtures in terms of thiosulfate. The figures obtained revealed that the optimum pH lies at 4.5.

Next the progress of cleavage at the optimum pH was followed up on 15.6 cc. of an incubation mixture in a similar proportion of the components. The results are embodied in Table II. k became less and less with the lapse of incubation time which suggested the occurrence of a reverse reaction.

TABLE I

Cleavage of Desaminomucosin at Various pH's

Duration of incubation 2 hrs. Temperature 37°C.

pH of incub. mix.*	Decrease of thiosulfate† use cc.	Hydrolysis %
3.0	0.03	0.7
3.5	0.09	2.2
4.0	0.12	2.9
4.5	0.14	3.4
5.2	0.13	3.1
5.5	0.11	2.7
5.8	0.10	2.4

* Michaelis' tartrate buffer was used for adjustment to pH 3.0, and Sörensen's citrate solutions for buffering at the other pH's.

† 0.005 N solution.

TABLE II

Cleavage of Desaminomucosin by the Snail Enzyme at pH 4.5

Incubation temperature 37°C.

Time of incubation min.	Decrease of thiosulfate* use cc.	Hydrolysis %	k† $\times 10^3$
30	0.04	1.0	0.34
60	0.08	1.9	0.32
60× 2	0.14	3.4	0.29
60× 3	0.19	4.6	0.26
60× 4	0.24	5.8	0.25
60× 5	0.28	6.8	0.25
60× 8	0.34	8.2	0.21
60×10	0.42	10.1	0.18

* 0.005 N solution.

† $k = \frac{1}{t_{min}} \times \ln \frac{100}{100\text{-}\%\text{cleavage}}$.

Separation of Glucuronic Acid from the Digestion Products of Desaminomucosin

Desaminomucosin as the material. At first, free mucosin was prepared as described in the First Report.[5] Then, 4 g. of it were deaminized as accounted for in the Second Report[2] by treatment with $AgNO_2$ three times and 850 mg. of a nearly nitrogen-free substance (N 0.04%) were obtained. The amorphous product gave the positive Goldschmiedt and Neuberg-Saneyoshi reaction. Molisch and Elson-Morgan, almost negative.

Analysis:

Ash.

12.65 mg. substance, 0.31 mg. ash (2.50%).

Iodine use as glucose (Macleod and Robison).

6.91 mg. substance, 1.87 cc. 0.02 *N* iodine used.

Found 48.6% (49.8% on an ash free basis). Calc. for ash-free substance, 53.1%.

Glucuronic acid.

Indole method: 0.828 mg. substance (in 1 cc.), 0.48 mg. glucuronic acid. Found 58.0% (59.5% on an ash free basis).

Orcinol method: 0.552 mg. substance (in 1 cc.), 0.318 mg. glucuronic acid. Found 57.6% (59.1% on an ash free basis).

Calc. for ash free substance, 57.2%.

Separation of glucuronic acid as cinchonidine salt. The procedure of Nagaoka[6] was somewhat modified. 702 mg. of the material above were dissolved in 35 cc. of water. The solution, whose pH was 2.2, was adjusted to pH 4.5 by the aid of the saturated baryta solution (5 cc.) and after addition of 12 cc. of the enzyme solution employed above, placed in a thermostat of 37°C. The baryta solution was added from time to time to maintain the mixture at the same pH. After the lapse of 12 days, when 38.5% cleavage of the substrate had occurred, the mixture was freed from barium quantitatively with just sufficient H_2SO_4 (1 and 0.1 *N*), whereby the pH was lowered to 2.4. Then it was distilled in vacuo to 2 cc. (pH at this stage was 2.2) and precipitated with abs. alcohol (99%). To the centrifuged supernatant fluid was added the saturated $Ba(OH)_2$ till pH 9.2 and the occurring deposits were exhausted with changes of water (four 15 cc. portions). From the extracts combined (strongly alkaline) barium was again carried down as above. The centrifuged supernatant fluid was condensed by vacuum distillation to about 15 cc. (pH 2.2) and diluted with 10 cc. of methanol and 250 mg. of cinchonidine were added as dissolved in 20 cc. of methanol. The mixture here was kept in water at 50°C for 45 minutes (No coloration happened), followed by distillation in vacuo to dryness. The residual was stirred up with 30 cc. of water, the insoluble part was rejected by filtration and the filtrate was again distilled

and removed from most of the remaining insoluble part in a similar manner (water used, 30 cc.). Finally, the clear solution, concentrated to about 15 cc., was shaken with chloroform (two 70 cc. portions) to entirely eliminate the alkaloid and distilled to a very thick solution. Non-colored crystals in thin needles began to come out soon. After standing overnight in an ice-chest, the crystalline porridge was brought onto a suction funnel with a small quantity of ice-cold water. The crude product (F. P. 189–190°C) amounted to 75 mg. Recrystallized from alcohol, the substance melted sharp at 191°C and the mixed melting point test showed no depression.

Crystal water.

22.62 mg. substance lost 1.16 mg. H_2O at 100–110°C under reduced pressure.

$C_6H_{10}O_7 \cdot C_{19}H_{22}ON_2 + 1\frac{1}{2}H_2O$. Calc. 5.24%, found 5.13%.

N (Pregl's micro Kjeldahl method).

6.78 mg. of the anhydrous substance, 2.74 cc. 0.01 *N* acid neutralized by distilled NH_3.

$C_6H_{10}O_7 \cdot C_{19}H_{22}ON_2$. Calc. 5.74%, found 5.66%.

Equilibrium optical rotation in water.

$$[\alpha]_D^{\infty} = \frac{-0.96 \times 100}{1.301 \times 1} = -73.8°.$$

Through the Grant Committee of the Science Council was given a grant from the Education Department which enabled us to carry out this work.

H. M.

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The Tohoku Journal of Experimental Medicine, Vol. 55, No. 1, 1951

Pyruvic Acid and Heart Enlarging; and Acetaldehyde, Preliminary Report

By

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(Received for publication, November 16, 1951)

As stated in the preceding paper (Beriberi and Pyruvic Acid),[1] I started animal experimentation with freshly prepared pyruvic acid using rats fed on polished rice. At first I made a tentative experiment with 0.2% solution as in the case of methyl glyoxal,[2][3] but no effect whatever was seen. So I went to the length of using 10% solution in an amount of 0.5 cc. (which had been neutralized), but even then a specially deleterious effect was not seen. Even a decreasing body weight was not remarkable, so all the animals were to be killed on purpose. Not a single one showed ever a heart enlarging. As to the heart enlarging effect, there is no need of testing acetaldehyde on this point. But a large amount of acetaldehyde was identified in human milk. To make it surer, I also performed animal experimentation with acetaldehyde. 10% solution was used in an amount of 1 cc. in the case of rats and in an amount of 5 cc. in the case of young rabbits, all the animals having been fed on thiamine deficient food. All the animals were killed on purpose: in no case the heart was ever found enlarged.

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1) Sato, A. Tohoku J. of Exp. Med., 1951, **55**, 22. 2) Sato, A., and Takamatsu, A. ibid., 1934, **28**, 507. 3) Wako, H., Heart Enlarging Effect of Methyl Glyoxal and Experimental Production of Different Forms of Beriberi by the Poison, to be published in a forthcoming issue of this Journal.

* I am very grateful to associate Prof. Nakamura of Faculty of Chemistry in our University for assisting me in chemical work: further to Prof. Sano of Pediatrics for his helpful advice and sincere encouragement.

The Tohoku Journal of Experimental Medicine, Vol. 53, No. 1, 1951

Biochemical Studies on Carbohydrates

CXLVII. Heparin

By

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(Received for publication, June 10, 1951)

Masamune, Suzuki and Kondoh[1] have asserted that heparin is not a mucoitinpolysulfuric acid on the basis that it involves no acetyl in the molecule. According to them it is dextrorotatory, while mucoitin is levorotatory. This reveals that heparin desulfurylated i.e. its proper carbohydrate moiety can not be even deacetylated mucoitin, although shortening or lengthening of the chain of mucosin molecules would alter the direction of optical rotation. The iodine use (3.04 and 3.09% as glucose) of our two new preparations indicated that heparin has a less molecular weight than mucoitin (iodine use as glucose, 2.38%[2]) which is free from sulfuryl. And, on the other hand, the basic disaccharide unit, though separated in an amorphous state, diverged in optical rotation definitely from mucosin.

The basic disaccharide of heparin gave positive Elson-Morgan reaction, and hence is a glucosamine-glucuronide like mucosin. For distinction from this the writers designate it '*heparosin*.' Attempts to obtain a crystalline derivative of heparosin failed due to lack of enough material.

Experimental

Heparin as a Material

4.2 g. of commercial heparin,[3] which had been prepared from ox lung and contained nucleic acids but no biuret-giving substance, were dissolved in 70 cc. water and 10% $CuCl_2$ added cautiously until no more precipitation occurred (pH of the mixture was 4.6). The deposits were centrifuged and washed with 15 cc. of a dilute $CuCl_2$ solution at pH 4.6

three times with centrifugation. All the supernatant fluids were combined, distilled in vacuo to syrupy consistence and precipitated with 30 cc. of abs. alcohol. The deposits here were agitated with many changes of abs. alcohol containing 1% HCl to remove Cu, then with plain abs. alcohol to remove HCl and finally with abs. alcohol containing anhydrous sodium acetate to change heparin into acid sodium salt. After drying in a desiccator, it (900 mg.) was taken up in 60 cc. water and filtered, then, the filtrate was concentrated by vacuum distillation to about 1 cc. and precipitated with 10 volumes of abs. alcohol. Yield, 800 mg.—Prep. I.

By another run started with 2.2 g. of the crude material 0.5 g. of a product was given—Prep. II.

The purified substances gave neither biuret nor Fehling and were free from phosphorus. Molisch almost negative. Neuberg & Saneyoshi and Goldschmiedt, both positive.

Analyses are embodied in Table I.

TABLE I

Composition of the Heparin Preparations (Acid Sodium Salt)

Analysis	In per cent		In equivalents per equivalent weight	
	Prep. I	Prep. II	Prep. I	Prep. II
N	2.15	2.16	1.08	1.04
Glucosamine	25.54	26.56	1.00	1.00
Glucuronic acid				
By indole method	30.64	31.54		
By orcinol method	29.88	31.10		
Mean	30.26	31.32	1.09	1.09
Hydrolysable sulfur	10.80	11.05	2.37	2.33
Iodine use as glucose	3.04	3.09		
Ash	29.94	28.66		
Na	9.70*	9.28*	2.96	2.72

* Calculated from ash as sodium sulfate.

Judging from the figures obtained, the preparations were either a polymer of tetrasaccharide containing 5 sulfuric ester groupings[4] or mixtures of mono-, di- and tri-sulfate.[5]

Anticoagulative force. The procedure described by Utusi[6] was applied for the determination. Preps. I and II prolonged the coagulation time of human blood to 31′ 30″ and 40′ 0″ respectively at a dilution of 1:100,000. The control without heparin, 5′ 0″.

The Unit Disaccharide as Hydrochloride

1.9 g. lots of acid sodium salt of heparin, prepared as above, were

dissolved in 6 cc. of 10% HCl containing 1.62 g. of crystalline barium chloride and heated on a boiling water-bath under reflux. Barium sulfate began quickly to precipitate and the solution turned dark brown. After 1 hour, the mixture was cooled under tap water and filtered with suction. The bulk on the funnel was washed with three 15 cc. portions of hot water. And the filtrate and washings were removed from barium with H_2SO_4 as quantitatively as possible, avoiding the use of the acid in excess, and distilled in vacuo at first as such to about 10 cc. and then with 15 cc. of methanol to about 5 cc. three times. To the final condensate were added 5 volumes of ether. The flocculent precipitates were centrifuged, washed with ether and dried. The crude substance obtained (900 mg.) was taken up in 10 cc. of methanol and filtered and 6 volumes of dry ether added. The deposits, separated and dried, here (760 mg.) were dissolved in 2 cc. of water and carried down with 10 volumes of abs. alcohol and after washing with abs. alcohol, dried. 110 mg. of a very hygroscopic powder with brown shade were given—Prep. I. From the mother liquid were obtained 170 mg. substance by further addition of 20 cc. of abs. alcohol.

By a similar procedure starting with 0.5 g. of the acid heparin sodium salt were yielded 110 mg. of a product of the basic disaccharide as hydrochloride that corresponded to the above intermediate product amounting to 760 mg.—Prep. II.

If the materials were a mixture of heparins sulfurylated to various extents, the present products are not ruled out to have been a mixture of different disaccharides, but it is most plausibly assumed that even such different heparins as those possess a non-esterified carbohydrate skeleton common to all of them.

The preparations were Molisch-negative but Neuberg & Saneyoshi-, Elson & Morgan- and Goldschmiedt-positive. Hydrolysable sulfur was not detected. Chloride ion was present. They analyzed as shown in Table II.

The optical rotations in water were the following. It appeared no mutarotation did occur.

$$\text{Prep. I:}\quad [\alpha]_D^{15}=\frac{+0.54\times 100}{0.668\times 1}=+80.8°.$$

$$\text{Prep. II:}\quad [\alpha]_D^{15}=\frac{+0.59\times 100}{0.724\times 1}=+81.5°.$$

For comparison's sake, the analytical values and rotatory force of two preparations of mucosin hydrochloride obtained from mucoitin in a similar manner will be given as Table III.

Conclusions

1. Heparin which contains sulfuric ester groupings is of lower molecu-

TABLE II

Composition of the Basic Disaccharide Unit as Hydrochloride of Heparin

Analysis	In per cent		In equivalents per equivalent weight	
	Prep. I	Prep. II	Prep. I	Prep. II
N	3.80	3.65	1.09	1.01
Glucosamine	44.43	46.20	1.00	1.00
Glucuronic acid				
By indole method	52.20			
By orcinol method	51.50			
Mean	51.85		1.08	
Reduction of ferricyanide as glucose	42.71		0.96	
Ash	2.86	1.98		

TABLE III

Analyses and Optical Rotation of Preparations of Mucosin Hydrochloride

	Prep. I	Prep. II
	Analyses as percentages	
N	3.48	3.40
Glucosamine	45.62	44.70
Ash	1.34	1.62
	Optical rotation in water	
$[\alpha]_D^{t}$ (in water)	$\frac{+0.55\times 100}{2.063\times 1} = +26.7°$ (20°C)	$\frac{+0.27\times 100}{0.987\times 1} = +27.4°$ (22°C)

* No mutarotation was observed.

lar weight than mucoitin that is non-esterified.

2. The basic disaccharide unit of heparin is different from mucosin. We designate it '*heparosin*.'

Through the Grant Committee of the Science Council was given a grant from the Education Department in aid to us. H. M.

References and Notes

1) Masamune, Suzuki and Kondoh, J. Bioch., 1940, **31**, 343.

In Bibliography and Notes, 6) of Utusi's article (Tohoku J. Exp. Med., 1949, **51**, 217), one of the writers mentioned that one of the sulfate groups of heparin, which had been considered to take the type of esteramide -NH-SO_2-OCH-, had been possibly a simple amide -NH-SO_2OH because we had overlooked the possibility that the heparin salt examined had been an

acid salt.

Cf. also Jorpes, Boström and Mutt, J. Biol. Chem., 1950, **183**, 607.

2) Osaki and Hasegawa, Tohoku J. Exp. Med., 1943, **45**, 176.

3) The writers with to thank Nakamura Taki Pharmaceutical Co. for the gift of this material.

4) Cf. Charles and Todd, Bioch. J., 1940, **34**, 112.

5) Cf. Jorpes and Bergström, Bioch. J., 1939, **33**, 47; Jorpes and Gardell, J. Biol. Chem., 1948, **176**, 267.

6) Utusi, Tohoku J. Exp. Med., 1949, **51**, 101.

The Tohoku Journal of Experimental Medicine, Vol. 55, No. 1, 1951

Isolation and Identification of Methyl Glyoxal from Human Milk, Preliminary Report*

By

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(Received for publication, November 16, 1951)

Soon after the publication of Arakawa's reaction[1] from our Laboratory Prof. Sato[2] was of the opinion that infantile beriberi was caused by methyl glyoxal (or like substance) in human milk, while he has since been doubting if pyruvic acid does ever play any all-important role in the disease.[3] But some pediatricians want to ignore his theory, as, since the Embden-Meyerhof scheme of carbohydrate metabolism became known, pyruvic acid has come into favor, while methyl glyoxal seems to have lost in favor. I am inclined to say that our Clinic has been the only one *to lay stress on methyl glyoxal* as the sole causative agent of the disease.

After a long continued discussion with associate Prof. N. Nakamura, esp. proficient in carbonyl chemistry, I was able to identify successfully methyl glyoxal from human milk under constant co-coperation of Prof. H. Tatsuta, another enthusiast in carbonyl chemistry.

1. Methyl glyoxal. Extracted from human milk, as methyl glyoxal bis-2;4-dinitrophenylhydrazone. M. P., 297°C, recrystallized by nitrobenzene:

$C_{15}H_{12}O_8N_8$	Theoretical	C=41.70%	H=2.80%	N=25.90%
	Found	C=41.98%	H=2.70%	N=26.07%

2. Acetaldehyde. Extracted from human milk as acetaldehyde-2;4-dinitrophenylhydrazone. M. P., 162°C, recrystallized by alcohol:

$C_8H_8N_4O_4$	Theoretical	C=42.90%	H=3.60%	N=25.00%
	Found	C=43.19%	H=3.31%	N=25.27%

3. Paper partition chromatography of human milk; methyl glyoxal: identified; acetaldehyde: identified. It was to our great surprise that pyruvic acid was not identified in my specimen of human milk from many lactants amounting to 1500 cc. and which was negative to Arakawa's reaction.

References

1) Arakawa, T., Tohoku J. Exp. Med., 1930, **16**, 107. 2) Takamatsu, A., and Sato, A., ibid., 1934, **28**, 506. 3) Sato, A., (Beriberi and pyruvic acid), ibid., 1951, **55**, 22.

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* The details will be published in a coming issue of this Journal.

I am very grateful to Prof. Sano of Pediatrics for his sincere encouragement and helpful advice.

The Tohoku Journal of Experimental Medicine, Vol. 53, No. 1, 1951

Biochemical Studies on Carbohydrates

CXLVIII. Structural Study of Group A-specific Carbohydrate from Mucus-Mucin of Pig Stomach

Seventh Paper

By

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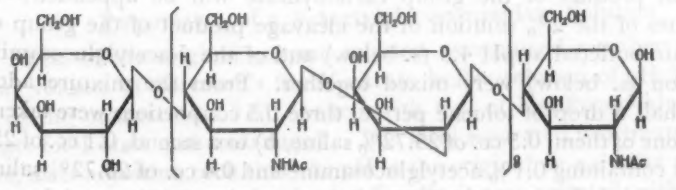
(Received for publication, June 10, 1951)

As to the proper carbohydrate moiety of the present group substance we[1] have disclosed that acetylglucosamine in one disaccharide unit combines C_{II} of galactose of the adjacent unit. In connection with the problem whether this union is α- or β-glucosidic, the writer first prepared a β-acetylglucosaminidase solution which contained β-galactosidase besides but almost no α-acetylglucosaminidase and, on the other hand, a partial-hydrolysis product (possibly a mixture of fragments) of the group carbohydrate by hot 0.1 N H_2SO_4. Treatment with the enzyme solution increased acetylglucosamine with bare C_I only scarcely in the original group carbohydrate, but markedly in the disintegration product. It seems most plausible that the enzyme solution involved little or no polyase for the group carbohydrate as such whereas it readily cleaves fragments of this carbohydrate by the alternative attack of β-galactosidase and β-acetylglucosaminidase (That the disaccharide unit is a β-galactoside has been already demonstrated[2]). On such a reasoning the writer believes that intermediate acetylglucosamine molecules partake in the make-up of the present group substance in β-type and the carbohydrate chain is expressed as:



Experimental

1) Analysis of Free Acetylglucosamine and Bound Acetylglucosamine with Bare C_I

The procedures of Osaki-Turumi[3] assay of acetylglucosamine and of Hamasato-Akakura[4] method for hexosamine analysis were combined as follows.

Reagents. A: $N/3$ Na_2CO_3 solution.
B: Reagent B of Hamasato-Akakura (a *p*-dimethylaminobenzaldehyde solution).

Procedure. 1 cc. of 12.86% saline solution that involves free acetylhexosamine or a carbohydrate having acetylhexosamine with bare C_I in the molecule and 1 cc. of Reagent A are mixed together in a test-tube (1.7 cm. × 17 cm.). The mixture is heated in a boiling water-bath exactly for 5 minutes and cooled without delay under tap water. Then after carefully overlaying 2 cc. of Reagent B and following agitation, it is again heated for 30 seconds and quickly cooled. Within 3–5 minutes after the last heating, the color development is compared against the standard which has been produced in parallel, starting with 0.5 mg. acetylglucosamine and set at 15 mm.

Calculation. The amount of acetylhexosamine or bound acetylhexosamine with bare C_I is read from a standardization curve plotted from the figures following:

Mg./cc.	Reading (mm.)
0.3	19.3
0.4	16.6
0.5	15.0
0.6	13.6
0.7	12.6
0.8	11.8
0.9	11.1
1.0	10.6

An example of quantitative recovery of acetylglucosamine added to a mixture of a β-acetylglucosaminidase solution and a partial disintegration product of the group carbohydrate will be appended:—Equal volumes of the 2% solution of the cleavage product of the group carbohydrate buffered at pH 4.5 (s. below) and of the β-acetylglucosaminidase solution (s. below) were mixed together. From the mixture added to with half a drop of toluene per cc. three 0.5 cc. portions were taken, and a) to one of them, 0.5 cc. of 25.72% saline, b) to a second, 0.1 cc. of 25.72% saline containing 0.1% acetylglucosamine and 0.4 cc. of 25.72% saline and c) to a third, 0.2 cc. of 25.72% saline containing 0.1% acetylglucosamine

and 0.3 cc. of 25.72% saline were added.

The recovery of acetylglucosamine by the present analytical method were 99% in b) and 100% in c).

	Reading mm.	Acetylglucosamine mg. per cc.	Recovery %
a)	13.7	0.595	
b)	12.65	0.694	99
c)	11.85	0.795	100

2) Determination of Optimum pH's for α- and β-Acetylglucosaminidase in the Enzyme Mixture of Snail Liver

With the enzyme stock porridge of snail liver, which had been kindly furnished by Mr. T. Isikawa, was prepared an enzyme solution (pH, 4.5) according to Utusi *et al*[5] (This will be referred to as 'the original enzyme solution'). And methyl-α-acetylglucosaminide (Freudenberg and others[6]), methyl-β-acetylglucosaminide (Neuberger and Rivers[7]) and phenol-β-acetylglucosaminide (Helferich and Iloff[8]) were incubated at various pH's with the original enzyme solution.

The incubation mixtures were composed of:

1 cc. of a buffer solution containing a substrate,

0.3 cc. of the enzyme solution which had been adjusted to respective pH's (HCl, CH_3COOH or NaOH was used) and filled up to a definite volume, and

1 drop of toluene.

Michaelis' acetate solutions were used as buffers from pH 2.0–3.0, and Sörensen's citrate solutions as those of pH's from 3.5 to 6.0. The substrate-buffer solutions were 0.042 molar with respect to methyl-acetylglucosaminides and 0.0135 molar with respect to phenol-β-acetylglucosaminide. Incubation temperature: 37°C. Duration of incubation was 24 hours for the methyl-acetylglucosaminides and 1 hour for the phenol-acetylglucosaminide. And immediately before and at the mentioned time after incubation 0.4 cc. was pipetted out from each of the mixtures to measure the iodine use according to Macleod and Robison.[9] The results illustrated in Table I indicate that the optimum pH's of the enzymes lie both at 4.5.

3) Preparation of a β-Acetylglucosaminidase Solution

30 cc. of the original enzyme solution were mixed together with an equal volume of Sörensen's 0.1 *N* citrate buffer solution of pH 3.0 (The pH of the mixture was 3.8), shaken with 6 g. of Kahlbaum aluminium silicate for 1 hour and centrifuged. The fluid separated was treated twice more, after adjusting each time the pH to the original, in a similar manner, using 6 and 3 g. of the adsorbent. Finally it was agitated with 3 g. of

TABLE I

Optimum pH's for α- and β-Acetylglucosaminidase of Snail Liver

pH of incubation \ Substrate	Methyl-α-acetylglucosaminide		Methyl-β-acetylglucosaminide		Phenol-β-acetylglucosaminide	
	Decrease of thiosulfate (0.005 N) use* cc.	Cleavage %	Decrease of thiosulfate (0.005 N) use* cc.	Cleavage %	Decrease of thiosulfate (0.005 N) use* cc.	Cleavage† %
2.0	0.0	0.0			1.04	6.98
2.5	0.09	1.72	2.00	38.17	2.70	16.52
3.0	0.27	5.15	2.72	51.91	3.90	23.87
3.5	0.41	7.82	3.04	58.02	4.62	29.50
4.0	0.48	9.16	3.32	63.36	4.96	30.35
4.5	**0.50**	**9.54**	**3.36**	**64.12**	**4.98**	**30.48**
5.0	0.49	9.35	3.28	62.60	4.80	29.38
5.5	0.39	7.44	3.00	57.25	4.40	26.93
6.0	0.24	4.58			2.46	15.06

* Expresses the corresponding increase of iodine use.

† The iodine use by liberated phenol is taken into account for the calculation.

TABLE II

Potencies of α- and β-Acetylglucosaminidase and β-Galactosidase in the Enzyme Solution Treated with Aluminium Silicate

Time of incubation (hrs.) \ Substrate	Methyl-α-acetylglucosaminide		Methyl-β-acetylglucosaminide		Lactose	
	Decrease of thiosulfate (0.005 N) use* cc.	Cleavage %	Decrease of thiosulfate (0.005 N) use* cc.	Cleavage %	Decrease of thiosulfate (0.005 N) use* cc.	Cleavage %
1					1.75	28.09
6	0.0	0.0	0.37	21.76		
24	0.0	0.0	1.02	60.00		
24×3	0.07	4.12				

* Expresses the corresponding increase of iodine use.

the adsorbent without antecedent shift of the pH and filtered. To the filtrate was added 30% acetic acid up to a pH of 4.5 and preserved with 3 drops of toluene.

The enzymic potencies of acetylglucosaminidases and of β-galactosidase were estimated as described in 1), by incubating 1 cc. of the substrate-buffer solutions at pH 4.5 with 1 cc. of the enzyme solution and 1 drop of toluene and taking out 0.4 cc. from the mixtures from time to time for the analysis. Concentration of the substrates in the substrate-buffer solutions: methyl-α- and methyl-β-acetylglucosaminide, 0.021 molar; lactose, 0.0819 molar. As is demonstrated in Table II, almost no α-

acetylglucosaminidase was found in contrast with abundant β-acetylglucosaminidase and β-galactosidase.

4) Relationship between the Increases of Iodine Use and of Free Acetylglucosamine, when Methyl-β- and Methyl-α-acetylglucosaminide were Incubated with the Enzyme Solutions

2 cc. of a substrate-buffer (pH, 4.5) solution (molarity of the substrates: methyl-α-acetylglucosaminide, 0.042; methyl-β-acetylglucosaminide, 0.021) were incubated with 1 cc. of the original enzyme solution in one tube and with 2 cc. of the β-acetylglucosaminidase solution in another after addition of 2 drops of toluene. Incubation temperature, 37°C. A 0.5 and a 0.4 cc. portion respectively of the mixtures were employed for the determination of free acetylglucosamine and of iodine use. The figures in Table III which were obtained demonstrate that the increase of iodine use coincided with that of the amount of free acetylglucosamine, simultaneously revealing that no cleavage of N-acetyl linkage did occur (Cf. Table I of Masamune, Hiyama and Kobayasi's article[10]).

5) Partial Disintegration of the Group Carbohydrate with Acid

The group carbohydrate as a material was prepared as described in the First Paper (N 3.7%, galactose 44.2%, glucosamine 44.3%, acetyl 10.6%, ash 0.3%, iodine value 5.87). 1.3 g. of it were taken up in 13 cc. of 0.1 N H_2SO_4 and heated, as sealed, in a boiling water-bath for 2 hours. The solution, that had turned dark red, was neutralized, when cold, with the saturated $Ba(OH)_2$ to a pH of 5.8. The centrifuged supernatant fluid and the watery washing of the centrifugate were distilled together in vacuo to about 8 cc. and 10 volumes of 98% alcohol containing NaCl were added. The occurring precipitates were washed with 10 cc. of abs. alcohol and dried. 0.7 g. of a grey powder was yielded. N (micro Kjeldahl) 3.95 %; galactose 42.38% (indole method: 42.50%; orcinol method: 42.26%); glucosamine 43.22%; acetyl 9.46%; bound acetylglucosamine with bare C_I 12.9% (procedure in 1)); ash 1.66%; iodine value 13.10. The equivalent proportions of galactose : glucosamine : acetyl are calculated at 0.98 : 1.0 : 0.91.

6) Digestion by the Enzyme Solutions of the Group Carbohydrate and its Partial-disintegration Product

The composition of incubation mixtures

A. In the case of the group carbohydrate:

TABLE III

Relationship between Iodine Use and Amount of Free Acetylglucosamine, when Acetylglucosaminides were Incubated with the Enzyme Solutions

Enz. sol. / Substr. / Time of incubation (hrs.)	Original solution						β-Acetylglucosaminidase solution		
	Methyl-α-acetylglucosaminide			Methyl-β-acetylglucosaminide			Methyl-β-acetylglucosaminide		
	Free acetylglucosamine* %	Iodine use		Free acetylglucosamine* %	Iodine use		Free acetylglucosamine* %	Iodine use	
		Decrease of thiosulfate (0.005 N) use† cc,	Cleavage %		Decrease of thiosulfate (0.005 N) use† cc,	Cleavage %		Decrease of thiosulfate (0.005 N) use† cc.	Cleavage %
6	—	0.30	6.61	47.30	1.08	47.79	24.40	0.42	24.71
24	11.17	0.50	11.01	86.90	1.96	86.72	61.60	1.04	61.18

* Measured by the colorimetric method and expressed as percentages of total acetylglucosamine.
† Expresses the corresponding increase of iodine use.

i) 3 cc. of a substrate(2%)-buffer solution of pH 4.5,
1.5 cc. of the original enzyme solution,
1.5 cc. of the buffer solution of pH 4.5, and
3 drops of toluene.

ii) 3 cc. of a substrate(2%)-buffer solution of pH 4.5,
3 cc. of the *β-acetylglucosaminidase solution, and
3 drops of toluene.

B. In the case of the partial-distintegration product of the group carbohydrate:

i) Similar as in A, i);

ii) Similar as in A, ii).

Incubation temperature, 37°C.

Determination of free acetylglucosamine and bound acetylglucosamine with bare C_I together and of iodine use was effected on 0.5 and 0.4 cc. portions respsectively of the incubation mixtures.

The results are illustrated in Table IV.

TABLE IV

Digestion by Enzyme Solutions of the Group Carbohydrate and Its Split Product

Enz. sol. / Time of incubation (hrs.)	Original enzyme solution			β-Acetylglucosaminidase solution		
	Increase of free acetylglucosamine* %	Iodine use: Decrease of thiosulfate (0.005 N) use† cc.	Iodine use: Cleavage‡ %	Increase of free acetylglucosamine* %	Iodine use: Decrease of thiosulfate (0.005 N) use† cc.	Iodine use: Cleavage‡ %
A. *Digestion of the group carbohydrate*						
24	0.0	0.13	1.62	0.0	0.09	1.12
24×3	0.0	0.24	3.01	0.0	0.15	1.88
B. *Digestion of the split product*						
6	1.60 (1.38)§	0.87	10.89	1.10 (0.99)§	0.59	7.39
24	2.90 (2.60)§	1.36	17.02	2.30 (2.06)§	1.04	13.01
24×3	5.80 (5.19)§	1.87	22.29	4.60 (4.11)§	1.56	19.52

* Expresses the percentage of the substrate.

† Expresses the corresponding increase of iodine use.

‡ The iodine use in terms of per cent of the corresponding amount when all acetylglucosamine and galactose are liberated.

§ Expresses the molecules of acetylglucosamine liberated in per cent of the total hexose units in the substrate.

SUMMARY

1. A method for estimating free acetylhexosamine or bound acetylhexosamine with bare C_I was devised.

2. An enzyme mixture of snail liver (called 'the original enzyme solution') could not split acetyl from N-acetylglucosaminides.

3. Optimum pH's for α- and β-acetylglucosaminidase in it were found to be 4.5.

4. β-Acetylglucosaminidase could be separated from α-acetylglucosaminidase by adsorption of the latter to aluminium silicate. A β-acetylglucosaminidase solution, thus prepared, contained also β-galactosidase.

5. The original enzyme solution as well as the β-acetylglucosaminidase solution above could hydrolyze hardly the group carbohydrate from pig-stomach mucus but comparatively easily a partial-scission product of that carbohydrate with increase of the amount of free acetylglucosamine.

6. From the findings in 5. a conclusion was drawn that acetylglucosamine molecules in the inner part of the group carbohydrate chain take β-glucosidic type.

Our thanks are due to the Education Department, which gave a grant through the Grant Committee of the Science Council in aid to us.

H. Masamune.

References

1) Masamune and Yosizawa, Tohoku J. Exp. Med., 1950, **53**, 10; Yosizawa, ibid., 1951, **54**, 129.
2) Masamune and Yosizawa, ibid., 1949, **50**, 388; Yosizawa, ibid., 1950, **52**, 111.
3) Osaki and Turumi, ibid., 1947, **49**, 11.
4) Hamasato and Akakura, J. Bioch., 1941, **34**, 159.
5) Utusi, Huzi, Matumoto and Nagaoka, Tohoku J. Exp. Med., 1949, **50**, 175.
6) Freudenberg, Eich, Knoevenagel and Westphal, Ber. dtsch. chem. Ges., 1940, **73**, 447.
7) Neuberger and Rivers, J. Chem. Soc. London, 1939, 122.
8) Helferich and Iloff, Zeits. physiol. Chem., 1933, **221**, 252.
9) Macleod and Robison, Bioch. J., 1929, **23**, 517.
10) Masamune, Hiyama and Kobayasi, Tohoku J. Exp. Med., 1942, **44**, 35.

Note at proof-reading:—We have just detected a methylpentose in that group carbohydrate itself by descending paper chromatography. Nevertheless, the argument for the β-glucosaminide union above will still hold, until the presence of a particular polyase splitting such a fragment of the group substance as above is demonstrated. H. M.

The Tohoku Journal of Experimental Medicine, Vol. 53, No. 1, 1951

Biochemical Studies on Carbohydrates

CXLIX. Structural Study of Group A Carbohydrate from Mucus-mucin of Pig Stomach

Eigth Paper

By

Hajime Masamune and **Zensaku Yosizawa**
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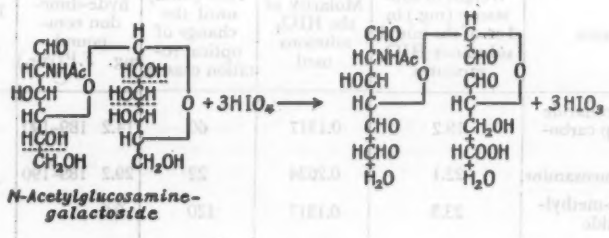
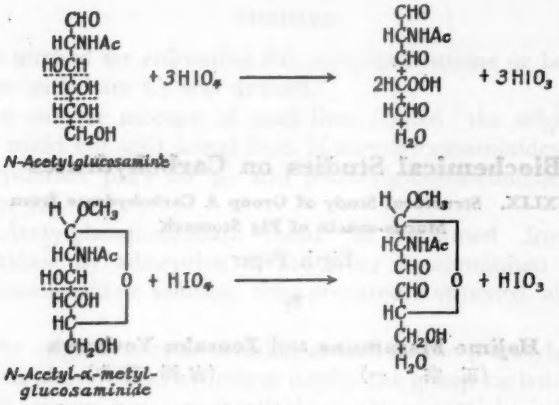
(From the Medico-chemical Institute, Tohoku University, Sendai)

(Received for publication, June 10, 1951)

In the Second Paper[1] on this topic it was stated that in the basic disaccharide unit of the present group carbohydrate, N-acetylglucosamine-β-galactoside, galactose combines either 4- or 5-position of acetylglucosamine, and in the Third Paper[2] the former possibility together with the pyranose form of the galactose molecule was ascertained by synthesis of the disaccharide started with lactose. Regretfully, however, both of the natural and synthetic products were not crystalline so that the mixed melting point test was not conducted daringly. The present account deals with a confirmation of the conclusion by separating the calculated amount of formaldehyde from the oxidation products of the natural substance by HIO_4. The material examined was from one and the same batch as the one in the Second Paper. As a control N-acetylglucosamine and N-acetyl-α-methylglucosaminide were also subjected to a similar procedure. The reaction equations (reproduced from the Second Paper):





Experimental

Method

Reeves method[3] was modified as follows. To 1.5 cc. of a solution of the substance to be analyzed and 3 cc. of a HIO_4 solution were united together and maintained at 20°C until the change of optical rotation stopped. Then, 3 cc. were pieptted out into a test-tube and after addition of 2 cc. of 8% $NaHCO_3$, allowed to stand again at the same temperature for 1 hour. Hereupon 3 cc. of 1 *N* HCl, 2 cc. of 1.2 *N* sodium arsenite, 2 cc. of 1 *M* sodium acetate and 1 cc. of the alcoholic solution containing 80 mg. dimedon per cc. of 95% alcohol were added in turn, and the test tube was placed for 10 minutes in a boiling water-bath, followed by standing for 1 hour at room temperature. The precipitates were filtered off on

Table I

Yields of Formaldehyde from the Basic Disaccharide of Group Carbohydrate Separated from Mucus-mucin of Pig Stomach (N-Acetylglucosamine-4-β-galactoside), N-Acetylglucosamine and N-Acetyl-α-methylglucosaminide

Substance	Weight of substance (mg.) in 3 cc. of the initial substance-HIO_4 mixtures	Molarity of the HIO_4 solutions used	Time (min.) until the change of optical rotation ceased	Formaldehyde-dimedon compound mg.	F.P.(cor.) °C	Formaldehyde % theor.
Basic disaccharide of the group carbohydrate	19.2	0.1317	60	14.2	189–190	97.3
N-Acetylglucosamine	22.1	0.2634	22	29.2	189–190	100
N-Acetyl-α-methylglucosaminide	23.5	0.1317	120	0.0		

a weighed filtros, well washed with water and dried for 20 minutes at 85-95°C.

Results

Table I illustrates the experiment and results.

Through the Grant Committee of the Science Council was given a grant from the Education Department in aid to us. H. M.

References

1) Yosizawa, Tohoku J. Exp. Med., 1950, **52**, 111.
2) Yosizawa, ibid., 1950, **52**, 145.
3) Reeves, J. Am. Chem. Soc., 1941, **63**, 1476.

The Tohoku Journal of Experimental Medicine, Vol. 55, No. 1, 1951

Methyl Glyoxal as Cause of Infantile Beriberi, Preliminary Report

By

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(Received for publication, November 16, 1951)

Arakawa's reaction[1] was published in 1930 from our Clinic. Human milk negative to Arakawa's reaction was defined as a milk from a thiamine deficient body. Soon after the publication of the reaction, it was noticed that Arakawa-negative milk is a more or less toxic milk. In 1934 Takamatsu[2] found that it contained methyl glyoxal (like substance) and showed with Sato[3] (one of the authors) that *synthetic methyl glyoxal* caused death with heart enlarging and hypernea in thiamine deficient animals, while thiamine was, as their results showed, able to keep the animals healthy in spite of use of the glyoxal.

We (Sato and Takamatsu) were thus able to publish as early as 1934[3] that (synthetic) methyl glyoxal would cause death with heart enlarging. Takamatsu[2] showed that methyl glyoxal could make Arakawa-positive milk Arakawa-negative, and in 1948 T. Morikawa[4] proved that Arakawa-negative milk could if treated with ether, become Arakawa-positive due to the removal of (ether-soluble) methyl glyoxal (like substance).

In spite of these and other investigations from our Clinic most of the beriberi references, busy with pyruvic acid, seem to have considered these works as unimportant[5] mainly because since the publication of the Emden-Mayerhof scheme of carbohydrate metabolism methyl glyoxal has been discarded.

Now strictly chemical investigation of human milk (negative to Arakawa's reaction) in a large amount was started by Wako (one of the authors), who was able to isolate methyl glyoxal[6] by strictly chemical analysis from it at last, while pyruvic acid was incapable of being identified from milk even by paper chromatography.[7]

In 1940, Fehily[8] experienced that, in Hongkong where fatal infantile beriberi had been prevailing, the milk of these mothers was Arakawa-negative, and that it was, as had been shown by many papers from our Clinic, changed to Arakawa-positive milk on thiamine administration to lactants.

The cause of acute cardiac death in infantile beriberi is methyl glyoxal, or beriberi death is due to methyl glyoxal poisoning.

References

1) Arakawa, T., Tohoku J. Exp. Med., 1930, **16**, 107. 2) Takamatsu, A., ibid., 1934, **23**, 46. 3) Takamatsu, A. and Sato, A., ibid., 1934, **28**, 506. 4) Morikawa, T., Is Human Milk Negative to Arakawa's Reaction Aperoxidatic Milk or Milk minus Peroxidase? (Preliminary Report). 229th Report of the Peroxidase Reaction. (176th Human Milk Study), to be published in a coming issue of this Journal. 5) For example, "Vitamin B," edited by Special Research Committee of Vitamin B (Japanese), Sogen-sha Tokyo, 1947, pp. 131–132. 6) Wako, H., "Isolation and Identification of Methyl Glyoxal from Human Milk, Parts I & II," to be published in a coming issue of this Journal. 7) Wako, H. Part III of the foregoing. 8) Fehily, L., The Caduceus (University of Hong-kong), 1940, **19**, 78.

Errata

Sato, A., and Wako, H., Methyl Glyoxal as Cause of Infantile Beriberi, Preliminary Report
This Journal, Vol. 55, No. 1. P. 46, 1951.
Line 15 *for* " Emden-Mayerhof " *read* Embden-Meyerhof.

The Tohoku Journal of Experimental Medicine, Vol. 55, No. 1, 1951

Biochemical Studies on Carbohydrates

CL. On Chondrosin and Chondroitinsulfuric Acid

By

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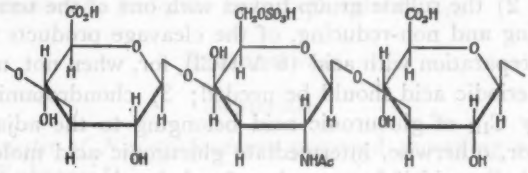
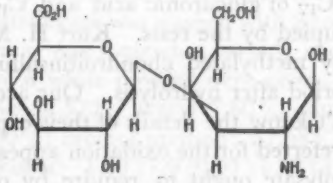
(Received for publication, June 10, 1951)

Kurt H. Meyer *et al*[1] assigned to chondroitinsulfuric acid the following formula:



Namely, regarding the structure of its basic disaccharide (chondrosin) they considered that glucuronic acid takes the pyranose form and glucosidically links with C_{III} of chondrosamine contrary to Kobayasi[2] from our Institute, who had put forward chondrosin to be chondrosamine-6-glucuronide on the basis that oxidation of it with mercuric oxide gives formylglucuronide. In order to decide the mode of union in question we subjected N-acetylchondrosinethylester to periodic acid oxidation and found 1 mole of formaldehyde is produced consuming 4 moles of periodic acid, which presented the formula below of chondrosin, in accord with the view of Kurt H. Meyer *et al.* in so far as the constitution of that basic



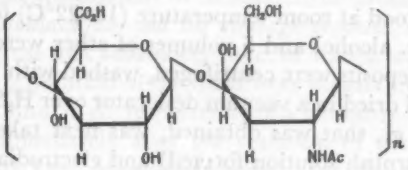
Chondrosin

disaccharide concerns. As a control experiment N-acetylchondrosamine and N-acetyl-α-methylchondrosaminide were examined as to the periodic acid consumptions under similar conditions. The results were the expected, that is, 3 moles and 1 mole respectively of periodic acid were used per mole.

In the next, chondroitinsulfuric acid (mol. w. 22300) and its two products (I, II) (mol. w.: I 12200, II 4100) obtained by partial degradation at the glucosidic and sulfuric ester linkages were investigated. All of these materials showed the positive indirect-Osaki & Turumi reaction distinctly and on the other hand, analysis gave the equivalent ratios of the aminosugar and hexuronic acid of 1:1. Therefore, acetylchondrosamine stood at the reducing end of the respective molecules and consequently glucuronic acid at the non-reducing. In the reaction with periodic acid, chondroitinsulfuric acid required 3 moles of the oxidizing agent per mole while the cleavage products 4 moles, which led us to the following conclusions: 1) in any of the substances, the inner part of the chain did not react at all; 2) the sulfate group linked with one of the terminal hexose units, reducing and non-reducing, of the cleavage products was cleft off during the preparation with acid (6 *N* HCl), for, when not, no more than 3 moles of periodic acid should be needed; 3) chondrosamine combines glucosidically C_{III} of glucuronic acid belonging to the adjacent acetylchondrosin, or, otherwise, intermediate glucuronic acid molecules would consume periodic acid if free or when freed, by the strong mineral acid, from the sulfate rest; 4) intermediate chondrosamine molecules are a pyranose, because, whether the sulfate rest is present or not, acetylchondrosamine bound at C_I and C_{III} is not oxidized at all in the pyranose form whereas the furanose would be, reducing 1 mole of the oxidizing agent if it is free from the sulfate rest or when the latter has been split off.

Nevertheless, from the above findings the sulfate groups can not be allocated to a definite position. Those rests can naturally combine any free position in the inner part of the chain of chondrosins without effect upon the HIO_4 consumption, since the mentioned part of the chain even in the state unattached by the rests is inert to the reagent, and moreover, chondroitinsulfuric acid can use 3 moles of the reagent when any one of the positions C_{II} and C_{IV} of glucuronic acid and C_{IV} and C_{VI} of acetylchondrosamine is occupied by the rests. Kurt H. Meyer and co-workers found that completely methylated chondroitinsulfuric acid had used 3 moles of HIO_4 per period after hydrolysis. Our acquaintance is through C. A. so that we don't know the details of their experiments. However, the conditions they preferred for the oxidation appear different from ours, because such a hydrolysate ought to require by our procedure only 2 moles of the reagent if the sulfate groups lie at C_{VI} of acetylchondrosamine

as they claimed. Be that as it may, where the sulfate groupings are situated is as yet an unsettled problem to us, and we content ourselves for the present with expression of chondroitin in the diagram:



Chondroitin

n amounts to 45–50. That the β-form is assigned to chondrosaminide is arbitrary. Cf. also Masamune & Osaki[3], Kobayasi[4], and Nagaoka.[5]

That Kobayasi obtained formylglucuronic acid after oxidation of chondrosin might be explained if one concedes the attack of mercuric oxide on the chondrosamine molecule from both sides of C_{III}.

Experimental

Materials

1) *Ethylester of N-acetylchondrosin* prepared according to Masamune Hiyama & Kobayasi.[6]

2) *N-Acetylchondrosamine.*

3) *N-Acetyl-α-methylchondrosaminide.*

These two were prepared as reported by Masamune, Maki & Hiyama.[7]

4) *Acid Na-salt of chondroitinsulfuric acid.* 2 g. of acid Na,Ca-salt of chondroitinsulfuric acid (Jorpes) were subjected, as dissolved in 100 cc. of water, to electrodialysis (200 volts) against changes of water, until no more shift of pH of the fluids in the outer compartments occurred, which took 2 days, and after rejecting some precipitates, the solution was neutralized with NaOH and concentrated to about 8 cc. The transparent yellowish condensate was precipitated with 10 volumes of abs. alcohol 1/4-saturated with sodium acetate. The centrifugate was washed with 20 cc. of abs. alcohol three times (The final washing was somewhat turbid) and thoroughly dried in a desiccator. 1.3 g. of a white powder were given. N (micro Kjeldahl) 2.63%, chondrosamine 32.57%, acetyl 7.60%, glucuronic acid 37.94% (indole method: 38.30%; orcinol method: 37.58%), hydrolysable sulfur 5.80%, (equivalent ratios: 1.03 : 1.0 : 0.97 : 1.07 : 1.0). Ash (Na_2SO_4) 21.82%. Iodine value 1.75. The molecular weight calculated from the iodine value, 24000 (22300 for free acid). Optical rotation in water:

$[\alpha]_D^{20} = \frac{-0.88 \times 100}{3.33 \times 1} = -26.4°$.

5) *Cleavage Product I of chondroitinsulfuric acid.* 20 cc. of a solution containing 2 g. of the material above and an equal volume of 10 *N* HCl were mixed and stood at room temperature (18–22°C) for 1 day, and then 10 volumes of abs. alcohol and 5 volumes of ether were added. The occurring purplish deposits were centrifuged, washed with two 40 cc. portions of abs. alcohol and dried in a vacuum desiccator over H_2SO_4. The powder amounting to 1.4 g., that was obtained, was next taken up in 60 cc. of water (A clear purplish solution formed) and electrodialyzed as above for 3 days followed by rejecting some precipitates and distilling in vacuo to about 5 cc., to which abs. alcohol and ether were added similar to above. The deposits were dried in vacuo over H_2SO_4 at first and afterwards over P_2O_5. 1.15 g. of a greyish powder were yielded. N (micro Kjeldahl) 3.60%, chondrosamine 44.96%, acetyl 10.32%, glucuronic acid 49.0% (indole method: 49.57%; orcinol method: 48.43%), hydrolysable sulfur 3.11%, (equivalent ratios: 1.03 : 1.0 : 0.96 : 1.01 : 0.39). Ash 0.0%. Iodine value 3.45. The molecular weight calculated from the iodine value, 12200. $[\alpha]_D^{20} = \frac{-0.35 \times 100}{1.44 \times 1} = -24.3°$ (in water).

6) *Cleavage Product II of chondroitinsulfuric acid.* 2 g. of chondroitinsulfate (4)) were dissolved in 40 cc. of water and added to with 60 cc. of 10 *N* HCl. The mixture was allowed to stand for 3 days at room temperature (18–20°C) and precipitated with 9 volumes of abs. alcohol and 4 volumes of ether. The deposits were centrifuged, washed with two 30 cc. portions of abs. alcohol and dried over H_2SO_4. 1.2 g. was the powder obtained. This was subjected to electrodialysis after dissolving in 80 cc. of water (a clear purplish solution) for 3 days and some appearing precipitates centrifuged off. The supernatant fluid was condensed and precipitated and the precipitates desiccated, as above. A greyish powder weighing 0.8 g. was the product. N (micro Kjeldahl) 3.63%, chondrosamine 45.40%, acetyl 10.27%, glucuronic acid 50.14% (indole method: 50.67%; orcinol method: 49.60%), hydrolysable sulfur 0.84%, (equivalent ratios: 1.02 : 1.0 : 0.94 : 1.02 : 0.10). Ash 0.0%. Iodine value 10.17. The molecular weight calculated from the iodine value, 4100. $[\alpha]_D^{20} = \frac{-0.28 \times 100}{1.38 \times 1} = -20.3°$ (in water).

Methods of Oxidation and Separation of Formaldehyde

The methods described by Yosizawa[8] and by Masamune and Yosizawa[9] were adapted.

N-Acetylchondrosinethylester, N-acetylchondrosamine and N-acetyl-α-methyl-

chondrosaminide. 5 cc. of 0.1 *M* solution of a substance and 10 cc. of a HIO_4 solution were mixed and let react at 20°C until the change of optical rotation stopped. Thereupon, 3 cc. portions were pitpetted out to measure the excess of HIO_4 and the amount of formaldehyde produced.

Acid-Na-chondroitinsulfate and the cleavage products I and II. 50 cc. of 3.01% solution of the first substance, 10 cc. of 4.33% solution of the second and 10 cc. of 4.13% solution of the third were maintained at 20°C together with 10 cc., 20 cc. and 20 cc. respectively of a variously concentrated HIO_4 solution. And immediately after the cessation of the change of optical rotation 12, 6 and 3 cc. respectively were taken out of the mixtures for determination of the excess HIO_4.

Results

See Table I.

TABLE I

Requirements of Periodic Acid by N-Acetylchondrosinethylester, N-Acetylchondrosamine, N-Acetyl-α-methylchondrosaminide and Chondroitinsulfuric Acid (Acid Sodium Salt), and Its Cleavage Products, and Productions of Formaldehyde from the First Three

	N-Acetyl-chondros-inethyl-ester	N-Acetyl-chondros-amine	N-Acetyl-α-methyl-chondros-aminide	Chondroitinsulfuric acid		
				Acid Na-salt	Cl. Prod. I	Cl. Prod. II
Requirements of HIO_4						
Molarity of HIO_4 sol. added	0.2634	0.2634	0.1317	0.0436	0.0218	0.0436
Time (min.) till cessation of the change of opt. rotation	6×60	21	35	45	60	70
Moles of the excess HIO_4 in total	0.123 $\times\frac{5}{1000}$	0.214 $\times\frac{5}{1000}$	0.151 $\times\frac{5}{1000}$	0.046 $\times\frac{5}{1000}$	0.058 $\times\frac{5}{1000}$	0.046 $\times\frac{10}{1000}$
Mols of HIO_4 required per mole	4.04	3.13	1.12	3.29	4.11	4.09
Yields of formaldehyde						
Mg. substance in 3 cc. of the oxidation mixtures	44.3	22.1	24.4			
Mg. formaldehyde-dimedon compound (ppt.)	28.2	28.7	0.0			
F.P. of ppts. (cor.) °C	189–190	189–190				
HCHO recovered, % theor.	96.6	98.3	0.0			

SUMMARY

N-Acetylchondrosinethylester, and a chondroitinsulfate and its partial

cleavage products were allowed to react with HIO_4 and the requirements of the oxidizing agent per mole of them and also the production of formaldehyde from the first one were estimated. The findings thereby and else suggested that 1) chondrosin is chondrosamine-3-β-glucuronide, and 2) chondroitin is expressed by Fig. 3 with acetylchondrosamine at the reducing end of the chain and glucuronic acid at the non-reducing.

Through the Grant Committee of the Research Council was given a grant from the Education Department which enabled us to carry out this investigation.

H. M.

References

1) Meyer, Kurt H., Odier and Sigrist. Cited in Chem. Abs., 1949, **43**, 148.
2) Kobayasi, Tohoku J. Exp. Med., 1947, **49**, 77.
3) Masamune and Osaki, ibid., 1943, **45**, 121.
4) Kobayasi, ibid., 1947, **49**, 84.
5) Nagaoka, ibid., 1950, **53**, 29.
6) Masamune, Hiyama and Kobayasi, ibid., 1942, **44**, 33.
7) Masamune, Maki and Hiyama, ibid., 1951, **54**, 313.
8) Yosizawa, ibid., 1950, **52**, 111.
9) Masamune and Yosizawa, ibid., this volume, p. 43.

The Tohoku Journal of Experimental Medicine, Vol. 55, No. 1, 1951

Estimation of Nucleic Acids in Microorganisms

(First Report)

On the Estimative Observation of Nucleic Acids in Salmonella abortivo-equina

By

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(Received for publication, June 11, 1951)

Two ways are known to estimate the nucleic acids as follows:—One is to try directly by means of staining the nucleic acids with specific dyes histochemically,[1] the other is to determine characteristic constituents of each nucleic acid which are separated from nucleic acids by various methods. The latter consists of two main methods, namely either to estimate ribose and desoxyribose or to estimate phosphor.

The former was reported by Schneider[2] and not to separate ribonucleic acid (RNA) or desoxyriboucleic acid (DNA) from each other, but to determine spectrophotometrically each of both sugars in mixture by specific colour reactions after the deproteinization with hot trichloroacetic acid.

In the latter the substrate was treated with relatively strong alkali, and under this condition RNA is transformed into acid-soluble compounds, whereas DNA is quantitatively precipitated on acidification of the alkaline hydrolysate. Schmidt and Thannhauser[3] estimated the amount of each of nucleic acids by the determination of phosphor separated into two fractions mentioned above.

In the method of Schneider, he used orcinol, diphenylamine and carbazole colour reactions. If the extracted samples contain the protein or polysaccharide-compounds, however, they will make difficult this colorimetric estimation of nucleic acids, especially for our unsufficient apparatus and equipments; besides the different resistance of purine- and pyrimidine-nucleotides toward hydrolysis presented another difficulty for the same purpose, as it arrested attention of Levene et al.,[4] and furthermore, as it was stressed also by Chargaff and Vischer,[5] Schmidt[6] and Schneider.

The author estimated the nucleic acids of *Salmonella abortivo-equina*

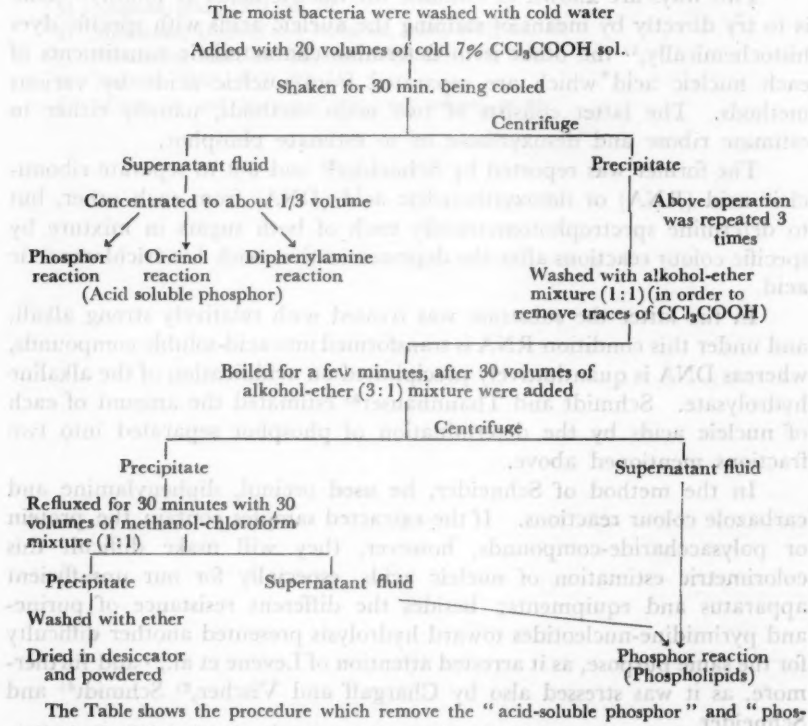
in various growth stages with the combined and somewhat modified method of these two different procedures.

Although the estimation of the nucleic acid amounts of various bacteria has been done already in detail by Boivin, Vendrely, Leonardi[7] and Stephenson,[8] the author estimated these of *Salmonella abortivo-equina* in order to prepare for the further studies to estimate the nucleic acids in various bacteria in various conditions.

Experimental

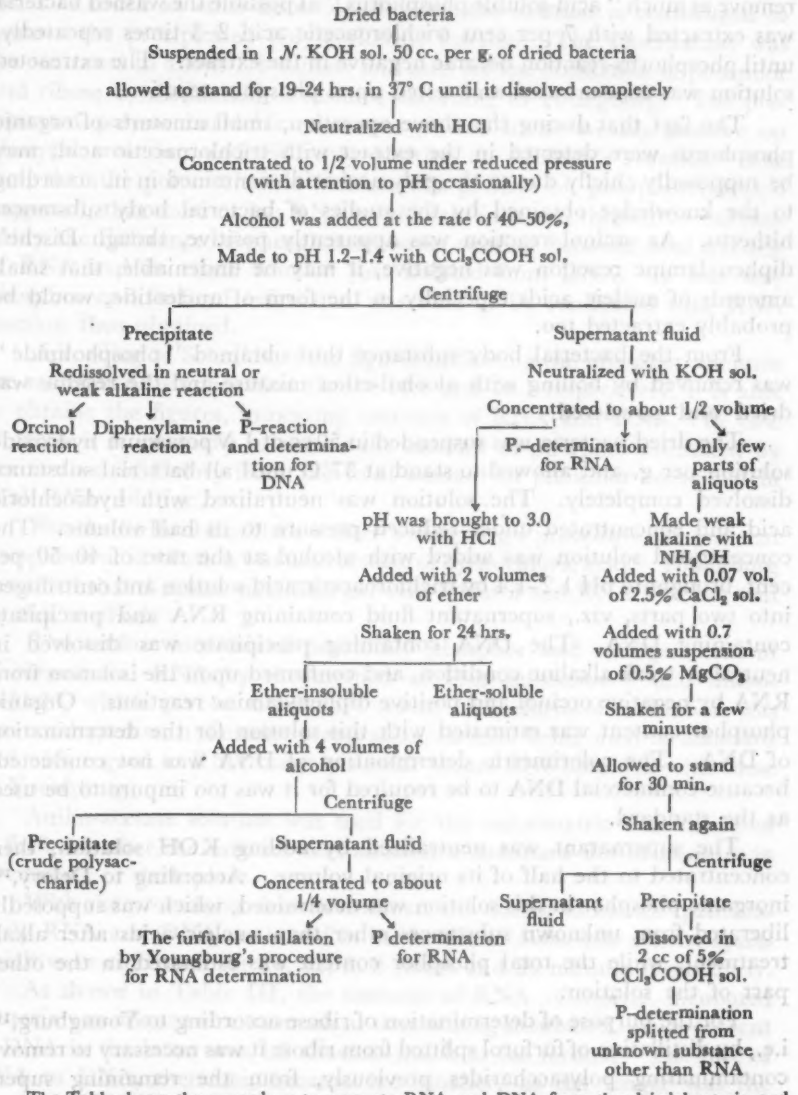
A strain of Salmonella abortivo-equina was cultivated in broth medium at 37°C by shaking during different hours, then formalin was added into it at rate of 0.5 per cent. After several hours the bacteria were washed with cold water in order to remove the broth constituents after Levy.[9]

Table I


The moist bacteria were washed with cold water
Added with 20 volumes of cold 7% CCl_3COOH sol.
Shaken for 30 min. being cooled
Centrifuge
Supernatant fluid
Precipitate
Concentrated to about 1/3 volume
Above operation was repeated 3 times
Phosphor reaction
Orcinol reaction
Diphenylamine reaction
(Acid soluble phosphor)
Washed with alkohol-ether mixture (1:1) (in order to remove traces of CCl_3COOH)
Boiled for a few minutes, after 30 volumes of alkohol-ether (3:1) mixture were added
Centrifuge
Precipitate
Supernatant fluid
Refluxed for 30 minutes with 30 volumes of methanol-chloroform mixture (1:1)
Precipitate
Supernatant fluid
Washed with ether
Dried in desiccator and powdered
Phosphor reaction (Phospholipids)

The Table shows the procedure which remove the "acid-soluble phosphor" and "phospholipides." This is done in a low temperature under 5C at least, which was required for the extraction of "acid-soluble phosphor" from the bacteria, as Levy described.

TABLE II


Dried bacteria
Suspended in 1 N. KOH sol. 50 cc. per g. of dried bacteria
allowed to stand for 19–24 hrs. in 37° C until it dissolved completely
Neutralized with HCl
Concentrated to 1/2 volume under reduced pressure (with attention to pH occasionally)
Alcohol was added at the rate of 40–50%,
Made to pH 1.2–1.4 with CCl_3COOH sol.
Centrifuge
Precipitate
Redissolved in neutral or weak alkaline reaction
Orcinol reaction
Diphenylamine reaction
P-reaction and determination for DNA
Supernatant fluid
Neutralized with KOH sol.
Concentrated to about 1/2 volume
P.-determination for RNA
Only few parts of aliquots
pH was brought to 3.0 with HCl
Added with 2 volumes of ether
Shaken for 24 hrs.
Ether-insoluble aliquots
Ether-soluble aliquots
Added with 4 volumes of alcohol
Centrifuge
Precipitate (crude polysaccharide)
Supernatant fluid
Concentrated to about 1/4 volume
The furfurol distillation by Youngburg's procedure for RNA determination
P determination for RNA
Made weak alkaline with NH_4OH
Added with 0.07 vol. of 2.5% $CaCl_2$ sol.
Added with 0.7 volumes suspension of 0.5% $MgCO_3$
Shaken for a few minutes
Allowed to stand for 30 min.
Shaken again
Centrifuge
Supernatant fluid
Precipitate
Dissolved in 2 cc of 5% CCl_3COOH sol.
P-determination splitted from unknown substance other than RNA

The Table shows the procedure to separate RNA and DNA from the dried bacteria and further to determine phosphor and furfurols isolated from the nucleic acids. The separation of two nucleic acids must be conducted as rapid as possible in a low temperature.

The further procedures are shown in Table I and II. In order to remove as much "acid-soluble phosphorus" as possible the washed bacteria was extracted with 7 per cent trichloroacetic acid 2–3 times repeatedly, until phosphorus-reaction became negative in the extract. The extreacted solution was concentrated and tested upon phosphor reaction.

The fact that during the above operation, small amounts of organic phosphorus were detected in the extract with trichloroacetic acid, may be supposedly chiefly due to the polysaccharide contained in it, according to the knowledge obtained by the studies of bacterial body substances hitherto. As orcinol reaction was apparently positive, though Dische's diphenylamine reaction was negative, it may be undeniable, that small amounts of nucleic acids especially in the form of nucleotide, would be probably extracted too.

From the bacterial body substance thus obtained "phospholipide" was removed by boiling with alcohol-ether mixture and the residue was dried and powdered.

The dried bacteria was suspended in 50 cc of 1 *N* potassium hydroxide solution per g. and allowed to stand at 37°C until all bacterial substance dissolved completely. The solution was neutralized with hydrochloric acid and concentrated under reduced pressure to its half volume. The concentrated solution was added with alcohol at the rate of 40–50 per cent, brought to pH 1.2–1.4 by trichloroacetic acid solution and centrifuged into two parts, viz., supernatant fluid containing RNA and precipitate containing DNA. The DNA containing precipitate was dissolved in neutral or weak alkaline condition, and confirmed upon the isolation from RNA by negative orcinol and positive diphenylamine reactions. Organic phosphor content was estimated with this solution for the determination of DNA. The colorimetric determination of DNA was not conducted, because commercial DNA to be required for it was too impure to be used as the standard.

The supernatant was neutralized by adding KOH solution, then concentrated to the half of its original volume. According to Delory,[10] inorganic phosphor in this solution was determined, which was supposedly liberated from unknown substances other than nucleic acids after alkali treatment, while the total phosphor content was estimated in the other part of the solution.

For the purpose of determination of ribose according to Youngburg,[11] i.e., by distillation of furfurol splitted from ribose it was necessary to remove contaminating polysaccharides previously, from the remaining supernatant. For this purpose the solution was brought to pH 3.0 with hydrochloric acid and after adding with ether twice as much as the solution, the mixture was shaken for 24 hours and separated into two layers; ether-

soluble and ether-insoluble. After adding with 95 per cent alcohol 4 times as much as ether-insoluble aliquots, the solution is centrifuged to remove the precipitated polysaccharide fraction. The supernatnat was concentrated to about 1/4 volume and used for the estimation of phorphor and ribose by distillation of furfurol according to Youngburg. The precipitated polysaccharide fraction was proved to be weakly positive on phorphor reaction and evidently positive on Molisch's reaction. The fact, that the polysaccharide fraction was positive on phorphor reaction suggested, that more or less RNA would exist in it and this will reveal probably the difference of phosphor amounts existing between final fraction of RNA and its crude fraction just isolated from DNA. In any way phosphor and furfurol estimation was able to be conducted with RNA fraction thus obtained.

According to Schmidt, if the figures indicating the phosphor amounts are multiplied by conversion factors 10.6 and 10.1 respectively, it is able to obtaine the figures, indicating amounts of RNA and DNA. In case of RNA it is necessary to reduce the amount of phorphor estimated by the method of Delory from the total amount of phosphor contained in final RNA solution.

For the furfurol distillation after Youngburg's procedure, 85 per cent phosphoric acid solution was used instead of hydrochloric acid. Youngburg described that d-xylose yield 64.1 per cent furfurol and RNA 10.4 per cent by this method.

The author ascertained previously phosphor and nitrogen content of commercial RNA by the method of Fiske-Subbarow[12] and micro-kjeldahl, which gave a satisfactory result. The furfurol estimation was also conducted with the same preparation, furfurol being used as standard and with regard to RNA roughly similar values were obtained with those of Youngburg.

Anilin-acetate solution was used for the colorimetric determination of furfurol, after the mixture was allowed to standard for 40 minutes in a semidark or dark place.

With the procedures described above, the author estimated quantitatively RNA and DNA contained in the bodies of *Salmonella abortivo-equina* which were cultivated for 24, 12, 6, 2 hours and 30 minutes respectively.

As shown in Table III, the contents of RNA of shortly incubated bacteria was about twice as much as that of long incubated, while content of DNA in the latter was 1.5 times as much as the former. The rate of RNA to DNA, therefore, became the smaller, as the longer was the incubation period.

TABEL III

Incubation periode (hrs)	Dried bacteria (g)	P-amounts by Delory (mg.)	RNA (%)		DNA (%)	RNA/DNA
			from P-amounts	from colour test of the sugar	from P.-amounts	
24	0.60	trace	6.2	6.0	3.9	1.5
12	1.05	"	6.8	7.8	3.3	2.2
6	1.17	"	6.6	7.3	3.2	2.1
2	1.01	0.3	13.7	15.0	2.1	6.8
30 min.	0.66	0.1	13.8	13.6	2.1	6.5

CONCLUSION

The author estimated quantitatively by combined and somewhat modified method of Schmidt and Schneider, RNA and DNA of *S. abortivo-equina* in various culture stages to establish the estimative method of nucleic acids of various microorganisms in various conditions and appearances.

The estimation of nucleic acids through carbohydrate component seems to be a very difficult problem owing to the existence of contaminating proteins or carbohydrates. However, the author has been able to remove polysaccharides as much as possible by the acidification of RNA containing solution in the presence of 4 volumes of alcohol for original solution. Moreover in the RNA containing solution it was possible by means of the distillation of furfurol to eliminate the influences owing to proteins and to exclude the consideration with regard to the difficulties of hydrolysis of purine— and pyrimidine-nucleotide.

Leonardi and others described that the total amounts of nucleic acids of microorganisms occupy about 8–20 per cent of the dry weight of bacteria and the author's experiment brought out a result nearly similar with it. Besides he reported that the protein synthesis of the bacteria was vigorous and the amount of RNA increased at the same time in early stage of incubation, namely in "lag phase."

Actually the bacteria in this phase seemed to be in a "growing" stage from morphological point of views. On the contrary, the fact that DNA increased in later stage of incubation, suggested that it played an important role in the "multiplication" periode, viz. in so-called logrithmic stage, as Davidson[19] described, and this problem should be revealed by future investigations.

In conclusion I wish to express my sincere thanks to Professor M. Kuroya for his helpful guidances and his kind advices.

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The Tohoku Journal of Experimental Medicine, Vol. 55, No. 1, 1951

Nutritional Dystrophy with or without Macrocytic Anemia

Studies on the Nutrition of Children in Hirosaki Area

(9th Report)

By

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(Received for publication, June 16, 1951)

Angular stomatitis is very commonly found among the children in Hirosaki Area.[1] And papillae present mushroom like appearance and the tongue "magenta tongue."[2] If ariboflavinosis (or ariboflavinotic symptoms) is prevalent in this area, it is probable that some other factors of vitamin B group than riboflavin may also be more or less deficient.[3] On the other hand macrocytic anemia is not rare among children of the same area.

The first object of this investigation was to determine whether or not any relation ever existed between the incidence of macrocytic anemia and that of angular stomatitis among children who visit our Department. These children do not complain either of anemia or stomatitis at all, but they come to us on account of tuberculosis, ascarisiasis or other diseases.

Method and Materials

Peripheral blood from the ear lobe of sick children of the age of from 13 months to 14 years was examined for red cell count, hemoglobin content, hematocrit and white cell count.

Volume index was calculated from the standard table (Mugrage and Andresen)[4] for reds, hemoglobin and haematocrit at each age group.

Results and Comment

Among the 47 cases examined, angular stomatitis was found in 26 cases (55.3%) (Cf. Tables I and II). Hyperchromic macrocytic anemia was found in 9 cases (Cases 3, 5, 9, 11, 24, 25, 30, 31 and 34) and its distribution did not show a statistically significant difference between the group with angular stomatitis and that without it. Out of the 9 cases

TABLE I

Red Cell Count, Hemoglobin Content, Hematocrit, Color Index and Volume Index of the Sick Children with Angular Stomatitis

No. of cases	Date (1949)	Name	Age and sex	Diagnosis	Red cells (millions)	Hemoglobin (g %)	Color index	Hematocrit (%)	Volume index
1	13. V	S.S.	5y 3m ♂	Diarrhea	5.00	13.84	0.91	46	1.01
2	16. VI	I.N.	1y 9m ♀	Pertussis	5.60	12.98	0.79	37.5	0.78
3	20. VI	K.K.	1y 11m ♂	Convalescence of measles	4.30	17.3	1.38	46	1.20
4	22. "	U.M.	1y 9m ♀	Hepatosplenomegaly of unknown origin	3.70	11.24	1.04	33	1.04
5	1. VIII	S.K.	7y 11m ♂	Chronic diarrhea	3.00	12.97	1.41	36	1.31
6	15. "	M.K.	7y 10m ♂	Abdominal pain (Ascariasis ?)	4.50	13.84	1.07	34	0.83
7	19. "	S.K.	10y 0m ♀	Angina acuta	4.70	13.84	0.93	42	0.97
8	22. "	Y.H.	11y 5m ♀	Pleurisy with effusion	4.90	15.57	1.02	40	0.88
9	23. "	S.S.	4y 3m ♂	Tumor of the liver	3.90	13.84	1.17	44	1.24
10	27. "	K.M.	9y 6m ♂	Pharyngitis acuta	4.30	15.57	1.17	44	1.11
11	2. IX	M.S.	5y 3m ♂	Recurrent attacks of abdominal pain	3.20	11.24	1.16	50	1.61
12	16. "	T.N.	7y 2m ♀	Tuberculosis of the lungs	4.60	13.84	0.98	38	0.90
13	3. X	Ti.S.	10y 5m ♀	Ascariasis	4.70	16.43	1.13	46	1.06
14	3. "	S.I.	7y 4m ♀	Tuberculous peritonitis	4.80	15.57	1.06	39	0.80
15	3. "	K.I.	9y 10m ♀	Tuberculosis of trachesbronchial lymph nodes	4.00	15.57	1.32	41	1.11
16	14. XI	T.K.	9y 10m ♂	Nephrosis	4.55	13.4	0.95	40	0.95
17	16. "	T.K.	6y 7m ♂	Ascariasis	5.00	13.5	0.89	42	0.92
18	16. "	A.N.	12y 0m ♂	"	4.30	13.3	1.02	40	1.00
19	18. "	Y.A.	1y 4m ♂	Chronic diarrhea	6.60	13.5	0.74	42	0.74

20	21. »	H.S.	13y 4m ♀	Anorexia	4.50	13.5	0.96	44	1.05
21	25. »	M.I.	10y 6m ♂	Rheumatic fever	5.60	14.2	0.79	47	0.91
22	14. »	G.N.	2y 0m ♂	Tuberculosis of the lungs	5.00	13.84	0.95	40	0.90
23	19. »	S.I.	11y 2m ♂	Emaciation (Tuberculosis ?	4.50	13.1	0.82	46	1.10
24	21. »	Y.H.	7y 11m ♂	Anorexia	3.00	11.5	1.25	38.5	1.40
25	6. XII	E.M.	11y 9m ♀	Lymphadenitis colli tuberculosa	4.00	14.0	1.12	54	1.46
26	8. »	T.S.	5y 0m ♂	Dystrophia musculorum progressiva ?	5.00	14	0.93	46	1.01

TABLE II

Red Cell Count, Hemoglobin Content, Hematocrit, Color Index and Volume Index of the Sick Children without Angular Stomatitis

No. of cases	Date	Name	Age and sex	Diagnosis	Red cells (millions)	Hemoglobin (g %)	Color index	Hematocrit (%)	Volume index
27	6.VIII	O.N.	4y 5m ♂	Pharyngitis acuta	6.00	15.57	0.85	39	0.71
28	25. »	K.H.	3y 0m ♂	Tuberculosis of the lungs	5.00	12.97	0.87	45	1.00
29	23. »	H.N.	7y 7m ♂	Idiocy	5.00	12.97	0.85	51	1.11
30	27. »	Y.N.	6y 5m	Tvberculosis of the bronchial lymph nodes	3.20	13.84	1.43	38	1.31
31	30. »	Y.K.	1y 5m ♀	Heine-Medin's disease	3.80	12.97	1.24	54	1.66
32	31. »	A.M.	7y 2m ♂	Pharyngitis acuta	5.90	14.9	0.82	41	0.77
33	8. IX	Y.M.	1y 6m ♂	Hepatosplenomegaly	4.80	9.51	0.72	32	0.78
34	14. X	T.Y.	10y 7m ♀	Acrocyanosis	3.60	13.84	1.24	44	1.33
35	14. XI	K.T.	8y 1m ♀	Pleurisy with effusion	5.00	10.6	0.68	33	0.71
36	14. »	M.N.	4y 7m ♀	Oxyuriasis	4.20	13.3	1.02	39	1.02
37	15. »	T.A.	5y 0m ♂	Tuberculosis of the bronchial lymph nodes	4.75	13.8	0.96	39	0.90
38	15. »	K.H.	4y 3m ♂	Tuberculosis of the lungs	6.40	14	0.72	44	0.75
39	21. »	M.I.	6y 0m ♂	Heine-Medin's disease	4.20	13.5	1.06	33.5	1.14

40	21. »	H.O.	2y 1m ♂	Pleurisy with effusion	6.10	13	0.73	44.6	0.82
41	25. »	R.Y.	1y 3m ♂	Diarrhea	5.00	13.1	0.96	43	1.00
42	2. XII	H.S.	5y 0m ♂	Anorexia	5.60	13.0	0.77	42	0.83
43	5. »	S.I.	8y 5m ♂	Pleurisy with effusion	6.00	13.5	0.73	42.5	0.76
44	5. »	T.F.	13y 3m ♀	Beri-beri	5.00	13.5	0.87	43	0.92
45	5. »	M.K.	1y 1m ♀	Ulcerative glossitis	6.00	12.5	0.76	48.7	0.95
46	7. »	Y.S.	1y 10m ♀	Impetigo	4.10	12.2	1.02	46	1.26
47	10. »	F.N.	10y 2m ♂	Anorexia	6.30	13	0.66	38.5	0.66

TABLE III

Diagnosis and Clinical Notes of the Cases with Hyperchromic Macrocytic Anemia

No. of cases	Date	Name	Age and sex	Diagnosis	Clinical notes		
					Hyperpigmentation	Angular stomatitis	Anorexia
3	20. VI	K.K.	1y 11m ♂	Convalescence of measles	++	+	+
5	1. VIII	R.K.	7y 11m ♂	Chronic diarrhea	+	+	+
9	23. »	S.S.	4y 3m ♂	Tumor of the liver	+	++	?
11	2. IX	M.S.	5y 3m ♂	Recurrent attacks of abdominal pain	+	++	+
24	21. »	Y.H.	7y 11m ♂	Anorexia	+	+	+
25	6. XII	I.M.	11y 9m ♀	Lymphadenitis colli tuberculosa	−	+	+
30	27. »	Y.N.	6y 5m ♂	Tuberculosis of the bronchial lymph nodes	+	−	+
31	30. »	Y.K.	1y 5m ♀	Heine-Medin's disease	−	−	−
34	14. X	T.Y.	10y 7m ♀	Acrocyanosis	+	−	−

with hyperchromic macrocytic anemia, as will be seen from Table III, angular stomatitis was found in 6 cases (Cases 3, 5, 9, 11, 24 and 25), hyperpigmentation of the skin on the forehead, on the back of hands and on the dorsum of feet was noticed in 7 cases (Cases 3, 5, 9, 11, 24, 30 and 34), anorexia in 6 cases (Cases 3, 5, 11, 24, 25 and 30) and chronic diarrhea

in 2 cases (Cases 3 and 5).

It may thus be said that the sick children with hyperchromic macrocytic anemia were, in general, characterized by at least 2 symptoms of the following three:—hyperpigmentation, angular stomatitis and anorexia.

During the literature survey we came across Achar's[6] report dealing with "Nutritional Dystrophy." The term "Nutritional Dystrophy," advocated by Achar in 1950, seems to be more suitable for expressing the whole clinical picture of the children in question than the classical term "Infantile Pellagra," because "Nutritional Dystrophy" can include not only pellagra, but also other associated deficient conditions. But in our own cases the pigmentation was different from that of the classical pellagra. It was either a deeping of natural pigmentation (hyperpigmentation) of the skin on the back of hands, on the forehead and on the dorsum of feet, or mosaic skin. The actual cause of this deeping of natural pigmentation is not known, but is considered to be related closely to nutritional deficiency.[7]

On the course of further investigation on the clinical symptoms and blood pictures of this nutritional dystrophy, macrocytic anemia seemed to be more frequently met with in the children with pronounced hyperpigmentation than in those with lesspronounced hyperpigmentation.

Basing upon the most outstanding features among the manifestations of nutritional dystrophy, we tentatively divided this dystrophy into the following 3 forms:—(1) "pellagrous form" which is characterized by the skin lesions ranging from the classical pellagrous skin to deeping of natural pigmentation or mosaic skin; (2) "Edematous form" which is characterized by the edema due to hypoproteinemia unaccompanied by hyperpigmentation and (3) "angular stomatitis form" which is characterized by angular stomatitis alone.

Keeping these 3 clinical forms of nutritional dystrophy in mind, blood examinations have been started anew in cases which came to us later (Cf. Tables IV, V and VI).

As will be seen from Tables 4, 5 and 6, macrocytic anemia was found in 11 cases out of 15 cases of "pellagrous form," in all the 2 cases of "edematous form" and in 2 cases out of 5 cases of "angular stomatitis form." Hyperchromic macrocytic anemia was found in only 4 cases of "pellagrous form." These results are given diagrammatically in Fig. 1. It is to our great regret that in Achar's report above mentioned no description of blood picture was found.

Conclusions

Nutritional dystrophy (infantile pellagra) is prevalent among children who visit us with other complaints in Hirosaki Area: We classified it into

TABLE IV

Red Cell Count, Color Index, Volume Index and Mean Corpuscular Volume of the Children with the "Pellagrous Form" of Nutritional Dystrophy

No. of cases	Date (1950)	Name	Age and sex	Diagnosis	Red cells (millions)	Color index	Volume index	Mean corpuscular volume (μ^3)
1	14. XI	K.I.	3y ♂	Heine-Medin's disease	4.8	0.87	1.05	93
2	17. "	K.S.	2y 10m ♂	Infantile pellagra	4.1	0.9	1.04	92
3	7. X	S.S.	3y ♂	Heine-Medin's disease	4.5	0.9	0.99	88
4	6. IX	M.O.	6y ♂	Stomatitis aphthosa	3.45	1.15	1.3	125
5	14. "	T.K.	7y ♂	Chronic diarrhea	3.7	0.9	1.1	127
6	25. "	T.Y.	3y 5m ♂	Anorexia	4.5	1.0	1.02	91
7	26.VIII	T.I.	0y 7m ♀	Infantile pellagra	4.0	1.2	1.4	120
8	26. IX	M.S.	5y 6m ♂	Chronic diarrhea	3.55	1.0	1.21	133
9	16. X	Y.U.	4y 5m ♂	Tuberculosis of the lungs	4.0	0.93	1.10	100
10	20. "	J.N.	4y 11m ♂	Infantile diarrhea	3.0	1.05	1.12	101
11	21. "	M.M.	2y 11m ♂	Infantile pellagra	3.7	1.11	1.16	102
12	26. IX	T.N.	8y 8m ♂	Heine-Medin's disease	3.0	1.2	2.0	150
13	5. X	J.N.	1y 5m ♂	Diarrhea	4.3	1.0	1.18	100
14	5. "	Y.K.	4y 9m ♀	Rheumatic fever	3.2	1.14	1.24	112
15	20. V	H.Y.	10y ♀	Typical pellagra	3.7	1.02	1.76	100

TABLE V

Red Cell Count, Color Index, Volume Index and Mean Corpuscular Volume of the Children with the "Edematous Form" of Nutritional Dystrophy

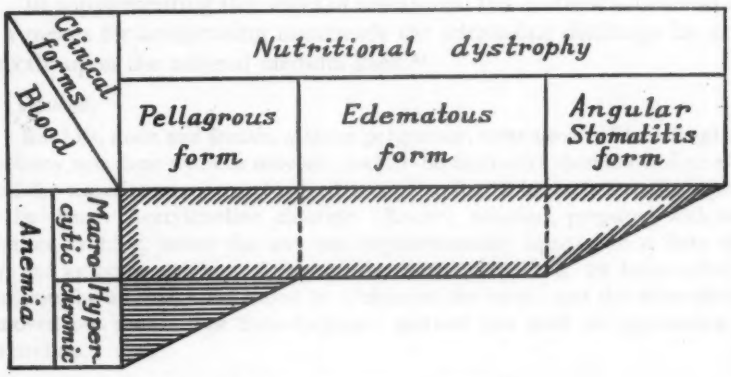
No. of cases	Date (1950)	Name	Age and sex	Diagnosis	Red cells (millions)	Color index	Volume index	Mean corpuscular volume (μ^3)
1	25. IX	Ts.S.	4y 1m ♂	Nutritional edema (Tuberculosis ?)	4.0	0.96	1.24	116
2	26. V	S.A.	2y 8m	Nutritional edema (due to poverty)	1.92	0.62	1.42	126

TABLE VI

Red Cell Color Index, Volume Index and Mean Corpuscular Volume of the Children with the "Angular Stomatitis Form" of Nutritional Dystrophy

No. of cases	Date (1950)	Name	Age and sex	Diagnosis	Red cell (millions)	Color index	Volume index	Mean corpuscular volume (μ^3)
1	14. XI	T.J.	3y 0m ♀	Bronchitis acuta	5.0	0.60	0.87	78
2	25. X	N.N.	10y 10m ♀	Ascariasis	4.5	0.89	1.11	105
3	25. "	A.T.	7y ♂	Tuberculosis of the lungs	6.2	1.0	0.87	79
4	9. "	J.K.	11m ♂	Diarrhea	5.0	1.09	1.07	94
5	20. XI	T.O.	6y ♂	Cheilosis	4.1	0.93	1.15	105

Fig. 1. Diagrammatical illustration of the relation between clinical forms and macrocytic anemia in nutritional dystrophy.



3 forms:—" pellagrous form," " edematous form " and " angular stomatitis form."

Macrocytic anemia seems to be more frequently found in both " pellagrous " and " edematous " forms than in " angular stomatitis " form of nutritional dystrophy.

Hyperchromic macrocytic anemia is found only in " pellagrous form."

This work was carried out by a Grant in aid for developmental scientific research of the Ministry of Education.

Prof. Ts. Arakawa.

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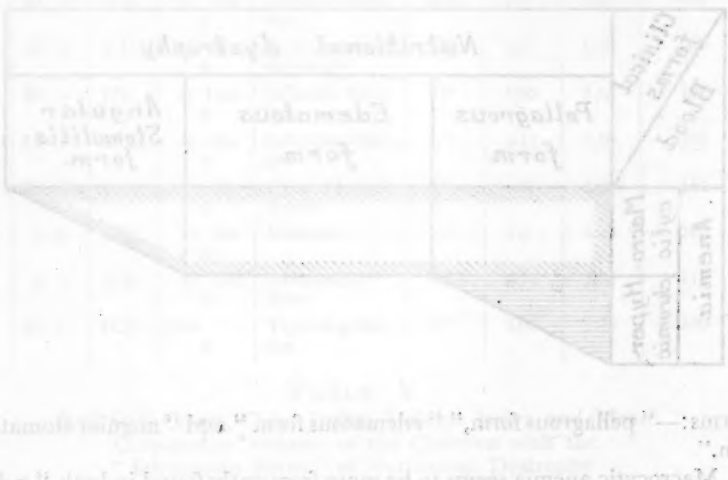

The Tohoku Journal of Experimental Medicine, Vol. 53, No. 1, 1951

Adrenaline Content of Adrenals in Rabbits Poisoned with Acetylcholine and its Recovery

By

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(Received for publication, June 19, 1951)

The previous researches rê the production of adrenaline in the adrenal gland were done to find whether the production is regulated by the nervous control and then to determine its velocity. The agencies which act to reduce the adrenaline content through the nervous route are: Diphtheria toxine on rabbits,[1] cold on cats,[2] physostigmine on dogs[3] and rabbits,[4] etc.

In supplementing this series of researches, the acethylcholine was used as a means for accelerating immensely the adrenaline discharge by acting directly upon the adrenal medulla itself.[5]

Method:

Rabbits, male and female without pregnancy, were used. The left splanchnicotomy was done without narcosis, and 21–68 days were then allowed to elapse until the experimentation. No food was given for 12 hours before the injection of the drug. Acetylcholine chloride (Roche) solution, prepared with saline solution, in 2.5% before the use, was hypodermically injected in a dose of 15 mg. per kg body weight. At certain intervals as 5, 12 & 24 hours after the injection the animals were killed by a blow on the neck, and the adrenals were removed out soon. The Suto-Kojima's method was used for estimating the adrenaline.

The data are given in the accompanying table.

As the control 9 rabbits were used.

On injecting the drug the animals suffered therefrom; the severe symptoms lasted ten to thirty minutes. Afterwards they looked quite normal.

As the consequence the glands taken out 5 hours after the injection were estimated extraordinarily heavy, as both glands together 341 mg. in toto and 165 mg. per kg b.w. as the average of 6 cases, against 211 mg. and 124 mg. per kg. in the 9 control cases. The glands taken out 12 hours and 24 hours respectively after the injection did not show such an increase in weight. This affords also an evidence against the use of adrenaline

TABLE

The Recovery of Adrenaline Content in the Denervated (Left) and in

No. af rabbits	Sex	L. splanchnicotomy			Interval between splanchnicotomy & experiment (days)	Experiment
		Date	Body weight (kg.)	Route		Date (1941)
						Normal
3	♀					12. III.
15	♀					19. IV.
18	♂					21. VI.
20	♂					28. VI.
45	♂					2. VIII.
46	♀					4. VIII.
47	♀					5. VIII.
48	♂					6. VIII.
49	♀					7. VIII.
	Mean					
						5 hours after the
1	♂	28. II. '41	1.76	abdominal	49	18. IV.
7	♀	5. IV. '41	2.28	"	30	5. V.
8	♀	11. IV. '41	1.42	"	31	12. V.
9	♂	11. IV. '41	1.40	"	41	22. V.
12	♂	7. V. '41	1.63	"	26	2. VI.
60	♀	8. IX. '41	1.46	"	22	1. X.
	Mean		1.66		33	
						12 hours after the
13	♂	7. V. '41	1.32	abdominal	66	13—14. VII.
16	♂	22. V. '41	1.59	"	58	19—20. VII.
19	♀	28. VI. '41	1.44	"	27	25—26. VII.
36	♂	26. VII. '41	2.07	"	25	20—21. VIII.
41	♂	27. VII. '41	1.45	lumbar	65	30. IX. — 1. X.
59	♂	8. IX. '41	1.34	abdominal	21	30. IX. — 1. X.
	Mean		1.54		44	
						24 hours after the
22	♀	11. VII. '41	2.00	abdominal	24	4—5. VIII.
24	♂	19. VII. '41	1.90	"	21	9—10. VIII.
25	♀	19. VII. '41	1.60	"	21	9—10. VIII.
34	♂	25. VII. '41	1.49	lumbar	26	20—21. VIII.
50	♀	7. VIII. '41	2.08	abdominal	21	28—29. VIII.
30	♀	21. VII. '41	1.42	lumbar	49	8—9. IX.
	Mean		1.75		27	

I

the Innervated (Right) Glands after the Exhaustion by Acetylcholine

Body weight (kg.)	Acetylcholine injection		Weight of gland (mg.)					
			L		R		L—R	
	Total quantity (mg.)	Duration of poisoning (min.)	Total	Per kg. body weight	Total	Per kg. body weight	Total	Per kg. body weight
control								
1.36			97	71	111	82	208	153
1.79			170	95	135	75	170	170
2.10			106	50	104	50	100	100
1.35			63	47	61	45	92	92
1.97			117	59	104	53	112	112
2.05			199	97	172	84	181	181
1.35			97	72	79	59	130	130
1.60			68	43	71	44	87	87
1.67			74	44	72	43	87	87
1.69			110	64	101	59	211	124
administration								
2.06	30.9	30	275	133	292	142	567	275
2.43	36.5	27	397	163	317	130	714	294
1.99	29.9	14	105	53	97	49	202	102
1.96	29.4	18	72	37	67	34	139	71
1.68	25.2	22	100	60	98	58	198	118
1.70	25.5	10	113	66	111	65	224	132
1.97	29.6	20	177	85	164	80	341	165
administration								
2.38	35.7	40	120	50	108	45	228	96
2.10	31.5	35	146	70	121	58	267	127
1.65	24.8	15	94	57	88	53	182	110
2.15	32.3	15	133	62	119	55	252	117
1.85	27.8	21	105	57	99	54	204	110
1.74	26.1	17	124	71	118	68	242	139
1.98	29.7	24	120	61	109	56	229	117
administration								
2.10	31.5	70	214	102	187	89	401	191
2.06	30.9	15	104	50	94	46	198	96
1.71	25.7	12	90	53	78	46	168	98
1.45	22.0	14	60	41	50	34	110	76
2.07	31.1	25	218	105	160	77	378	183
1.61	24.2	14	132	82	124	77	256	159
1.83	27.6	25	136	72	116	62	252	134

Continued on the back

No. of rabbits	Adrenaline content (mg.)									Room temperature (c)
	Total quantity			Per gr. of gland			Per kg. of body weight			
	L	R	L+R	L	R	L+R	L	R	L+R	
Normal control										
3	0.078	0.080	0.158	0.80	0.72	0.76	0.057	0.059	0.116	11
15	0.064	0.059	0.123	0.38	0.44	0.40	0.036	0.033	0.069	10
18	0.084	0.080	0.164	0.79	0.77	0.78	0.040	0.038	0.078	20
20	0.054	0.051	0.105	0.86	0.84	0.85	0.040	0.038	0.078	23
45	0.080	0.080	0.160	0.68	0.77	0.72	0.041	0.041	0.081	22
46	0.174	0.160	0.334	0.87	0.93	0.90	0.085	0.078	0.162	22
47	0.058	0.048	0.106	0.60	0.61	0.60	0.043	0.036	0.078	27
48	0.048	0.050	0.098	0.71	0.70	0.71	0.030	0.031	0.061	24
49	0.070	0.070	0.140	0.95	0.97	0.96	0.042	0.042	0.084	27
	0.079	0.075	0.154	0.74	0.75	0.74	0.046	0.044	0.090	
5 hours after the administration										
1	0.090	0.082	0.172	0.33	0.28	0.30	0.044	0.040	0.083	10
7	0.068	0.072	0.140	0.17	0.23	0.20	0.028	0.030	0.058	17
8	0.040	0.042	0.082	0.38	0.43	0.41	0.020	0.021	0.041	16
9	0.044	0.044	0.088	0.61	0.66	0.63	0.022	0.022	0.045	19
12	0.068	0.072	0.140	0.68	0.73	0.71	0.040	0.043	0.083	20
60	0.052	0.058	0.110	0.46	0.52	0.49	0.031	0.034	0.065	18
	0.060	0.062	0.122	0.44	0.48	0.46	0.031	0.032	0.063	
12 hours after the administration										
13	0.054	0.058	0.112	0.45	0.54	0.49	0.023	0.024	0.047	18
16	0.045	0.041	0.086	0.31	0.34	0.32	0.021	0.020	0.041	23
19	0.062	0.058	0.120	0.66	0.66	0.66	0.038	0.035	0.073	20
36	0.080	0.082	0.162	0.60	0.69	0.64	0.037	0.038	0.075	25
41	0.036	0.058	0.094	0.34	0.59	0.46	0.019	0.031	0.051	18
59	0.058	0.044	0.102	0.47	0.37	0.42	0.033	0.025	0.059	18
	0.056	0.057	0.113	0.47	0.53	0.50	0.029	0.029	0.058	
24 hours after the administration										
22	0.088	0.080	0.168	0.41	0.43	0.42	0.042	0.038	0.080	22
24	0.042	0.040	0.082	0.40	0.43	0.41	0.020	0.019	0.040	19
25	0.074	0.070	0.144	0.82	0.90	0.86	0.043	0.041	0.084	21
34	0.058	0.060	0.118	0.97	1.20	1.07	0.040	0.041	0.081	24
50	0.128	0.130	0.258	0.59	0.81	0.68	0.062	0.063	0.125	23
30	0.052	0.070	0.122	0.39	0.56	0.48	0.032	0.043	0.076	25
	0.074	0.075	0.149	0.60	0.72	0.65	0.040	0.041	0.081	

to the weight of gland as the adrenaline content in the case of rabbits.[6]

Then in consulting the figures of the total amount of adrenaline and the amount per kg. of b.w. on the table, the general outcome will be extracted in the following paragraphs:

In all the series of experiments the amount of adrenaline in toto and per kg. b. w. as well was almost same in the fellow glands, i.e. in the left denervated and the right intactly innervated. The loss of innervation has no influence upon either the decrease of adrenaline store by acetylcholine or its recovery.

Secondary the adrenaline content was smaller in the series of 12 hours after the injection than in that of 5 hours. This proves that the gland is still losing adrenaline at that period. It is however obscure when namely before or after 12 hours after the injection the adrenaline load diminishes at most. Safely can it be said that the gland restores one fourth of the normal store of adrenaline in first 12 hours.

In short, the adrenaline store of adrenals in rabbits is reduced by acetylcholine, without regard to whether the innervation of glands interferred with or not. The drug is fully proved to attack the adrenal medulla directly as reported by previous investigators.

The gland is able to recover the adrenaline load at the rate of one-fourth of the normal value in the second 12 hours after the injection of acetylcholine. The recovery process takes place independently of the innervation.

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The Tohoku Journal of Experimental Medicine, Vol. 55, No. 1, 1951

A Further Study on the Compensatory Increase of the Adrenaline Content in the Adrenal Gland in Rabbits

By

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(Received for publication, June 19, 1951)

While Stewart and Rogoff[1] and others, who saw a copious compensatory increase of adrenaline in rabbits, estimated it with the Folin method, Ooisi[2] applied the Suto-Kojima's corrosive sublimate method, and came to discover its non-occurrence. The former procedure measures besides adrenaline the ascorbic acid which is much involved in the cortical tissue, and this increases copiously in compensation.

In order to testify whether the data obtained in the hands of Stewart and Rogoff are due solely to the compensatory increase of the substance which is Folin-positive, but not adrenaline, some rabbits were experimented on with quite the same planning and procedures as Ooisi, only with an addition of the Folin method, modified by Kodama.[3]

The removal of glands through the lumbar way, without narcosis, consumed 30–56 minutes, and the I and II extirpation were separated by an interval of 85–166 days. 10 cc. of Folin's extract was prepared from one gland, and 5 cc. was used for the Folin-Kodama, and 4 cc. for the Suto, practized according to Ooisi. The matching was carried out with a micro-colorimeter of Dubosque type.

All the outcome are epitomized in the accompanying table. The left gland was removed out at first from 7 rabbits, and the right one from 4. The days, separating two removals, were similar in both the series of experiments, and the increase, in the body weight in those days, that is, 119 days in both series, was about 20 percent similarly. The gain in the second glands was clearly large in the latter series, where the left gland was remaining behind.

In the first place, the Folin value was found largely increased in the glands, staying behind. The total amount increased by 50–100%, and the value per kg. of body weight also by the same rate. They are comprehensivable. Now the fact that the concentration of the Folin in the re-

TABLE

Adrenaline Content in the Adrenals, Taken

Determination by the Suto

	Rabbits: No., sex	Date of I (Left) removal (1942)	Body weight (kg.)	I adrenal gland									Time spent for removal (Min.)
				Gland weight (g.)	Gl. weight per kg. b. w.	Adrenalin content (mg.) by Folin-Kodama			Adrenalin content (mg.) by Suto-Kojima-Ooisi				
						per gland	per g. of gland	per kg. of b. w.	per gland	per g. of gland	per kg. of b. w.		
L adrenal gland firstly removed	1	7 IX	2.20	0.376	0.170	0.222	0.590	0.101	0.089	0.237	0.040		40
	2	12 IX	2.24	0.240	0.107	0.125	0.521	0.056	0.056	0.233	0.025		40
	3	14 IX	2.23	0.203	0.091	0.166	0.817	0.047	0.047	0.231	0.021		36
	6	28 IX	2.06	0.146	0.071	0.091	0.623	0.044	0.052	0.356	0.025		45
	7	30 IX	2.50	0.311	0.124	0.177	0.569	0.071	0.056	0.180	0.022		38
	8	2 X	1.99	0.324	0.163	0.238	0.734	0.119	0.083	0.256	0.042		35
	9	6 X	2.36	0.222	0.094	0.232	1.045	0.098	0.089	0.401	0.038		35
		Min.	1.99	0.146	0.071	0.091	0.521	0.044	0.047	0.180	0.021		35
		Max.	2.50	0.376	0.162	0.238	1.045	0.119	0.089	0.401	0.042		45
		Mean	2.23	0.260	0.117	0.179	0.700	0.080	0.067	0.271	0.030		38
R adrenal gland firstly removed	7	23 X	1.91	0.214	0.112	0.217	1.014	0.114	0.085	0.397	0.045		55
	9	29 X	1.91	0.144	0.075	0.143	0.993	0.075	0.076	0.528	0.040		56
	11	21 XI	2.16	0.425	0.197	0.323	0.760	0.150	0.094	0.221	0.044		45
	12	24 XI	1.78	0.125	0.070	0.116	0.928	0.065	0.064	0.512	0.036		50
		Min.	1.78	0.125	0.070	0.116	0.760	0.065	0.064	0.221	0.036		45
		Max.	2.16	0.425	0.197	0.323	1.014	0.149	0.094	0.528	0.045		56
		Mean	1.94	0.227	0.114	0.200	0.924	0.101	0.080	0.415	0.041		52

maining left glands in the latter set was about the half of the normal, right glands seems strange. It might be however accounted for by the fact that the left glands in this set were more rapidly, increasing in comparison with those in the first group, as just above referred to. The tissue is growing rapidly, so that it is not yet filled up witht the acid.

In the second place, the data on the adrenaline, measurable by the Suto-Kojima method will be discussed now. Look at for a moment first on the data in the latter series of experiments: The total amount of adrenaline was measured as increased and in fact in paralled to the gain in he body weight. That is, the amount of adrenaline, reckoned on kg. body weight did not altered; about 0.041 and 0.043 they are. The concentration in the glands were estimated incomparatively thin; it might be however commented on the behalf of the copious enlargement of cortical

Out with Interval of Several Days

method and the Folin method.

Date of II (Right) removal (XII 42-43)		Body weight (kg.)	II adrenal gland								Time spent for removal (min.)	Interval between two operations (days)
			Gland weight (g.)	Gland weight per kg. b. w.	Adrenaline content by Folin-Kodama			Adrenaline content by Suto Kojima-Ooisi				
					per gland	per g. of gland	per kg. b.w.	per gland	per g. of gland	per kg. of b. w.		
1	XII	2.55	0.496	0.195	0.317	0.639	0.124	0.104	0.210	0.041	50	85
25	I	2.67	0.414	0.155	0.476	1.150	0.178	0.112	0.271	0.042	50	135
18	XII	2.64	0.344	0.131	0.312	0.907	0.118	0.120	0.349	0.045	45	95
21	I	2.34	0.510	0.218	0.250	0.490	0.107	0.093	0.182	0.040	55	115
15	III	3.02	0.580	0.192	0.266	0.459	0.088	0.096	0.177	0.032	36	166
29	I	2.39	0.703	0.294	0.588	0.836	0.246	0.125	0.178	0.052	40	119
4	II	2.97	0.590	0.199	0.571	0.968	0.192	0.110	0.186	0.037	40	121
		2.34	0.344	0.131	0.250	0.459	0.088	0.093	0.178	0.037	36	85
		3.02	0.703	0.294	0.588	1.150	0.246	0.125	0.349	0.052	55	166
		2.65	0.519	0.198	0.397	0.778	0.150	0.109	0.220	0.041	45	119
6	III	2.08	0.576	0.277	0.333	0.578	0.160	0.114	0.198	0.055	35	134
27	II	2.56	0.468	0.183	0.200	0.427	0.078	0.085	0.182	0.033	32	121
8	III	2.28	0.945	0.414	0.454	0.480	0.199	0.123	0.130	0.054	30	107
17	III	2.23	0.312	0.140	0.143	0.458	0.064	0.068	0.218	0.030	32	113
		2.08	0.312	0.139	0.143	0.427	0.064	0.068	0.130	0.030	30	107
		2.56	0.945	0.414	0.454	0.578	0.199	0.123	0.218	0.055	35	134
		2.29	0.575	0.254	0.283	0.486	0.125	0.098	0.182	0.043	32	119

tissue in these cases, as shown just above.

Turning now to the data in the former set: The figures rê the amount of adrenaline in toto and its concentration in the glands harmonize with the above statement, but the values of adrenaline, reckoned on the body weight, are definitely smaller in the glands, removed out at first. The figures are enough to let us second to the view of the compensatory increase of the medulli-adrenal tissue, although the data in the latter half of the present investigation and in the paper of Ooisi do not so. A closer investigation points us a number of cases with an abnormally smaller storage of adrenaline; 0.0225 mg per kg. b. w. is a very seldom occurrence.[4] If such rather abnormal cases be taken out of consideration, the figure of per kg of body weight can be arranged as 0.04 mg. Then this set of experiments does not run out of the general inclination. And in doing so,

the whole figures in the upper half of the table do not lose the harmony.

Briefly stated: In rabbits an adrenal gland was removed out, and four months later the fellow gland was excised in a similar manner. The gland, extirpated later, increased largely in weight, and the Folin positive substance showed a similar inclination quantitatively, but it was occasioned solely or at least chiefly by the substance, non-identified with adrenaline, namely by ascorbic acid.

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Occurrence of the Cold-puncture Hyperglycemia in the Dog

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Some twenty years ago Morita came to see an immense hyperglycemia in the rabbit, when it is rendered poikilothermic by sectioning the brain stem between the interbrain and midbrain, but somewhat wider than done by Leschke, and consequently the anal temperature falls below than 30°C.[1)]

In analyzing the mechanism whereby the cold-diabetes is brought about, Wada, Seo and Abe,[2)] and Satow[3)] detected in the dog an appearance of a conspicuous hyperglycemia only when the animal is cooled excessively so as to reduce the anal temperature below 30°C. or likely.

Now I have tried to unite both the experimental data by carrying out the cold-puncture in the dog and estimating the anal temperature and the blood sugar concentration simultaneously.

The puncture was done according to Morita[4)] and Hirayama,[5)] the instrument, devised by the latter, being somewhat magnified and modified. Under the local anesthesia with 0.5% procain a median skin incision was made on the parietal parts of the skull, and the muscle layer thereupon was cut off. About the middle between the junction of sutura coronalis and sutura sagitalis and proc. interparietalis, the skull is opened by means of a trepan, the sinus sagitalis sup. being ligated. The dura mater was transected in the breath of 12 mms. The circular washer was then fixed in the trepanized hole, and the sheath was adjusted to the proper direction, i.e. in the line connecting the proc. zygomat. ossis tempor. in both sides. On inserting the spatula, with a breath of 1 cm, the puncture or rather section was then carried out.

The protocol speaks for itself.

This case shows clearly the existence of coldpuncture hyperglycemia in the dog too, although here I have no change to fix the temperature, at which the blood sugar starts to increase with suddenness.

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TABLE

Dog Female

Time (hr.: min.)	Anal temp. (°C)	Room temp. (°C)	Blood sugar (%)	Respiratory rate	Heart rate	Procedures, symptoms, etc.
13. II 1943						7.3 kg. Both auricular nerves cut.
1. III						7.3 kg. R. fem. a. uncovered, a cannula
9:30	38.3	12	0.100	18	94	inserted, for collecting blood. Sugar: Ha-
10:00	38.3	14	0.098	20	98	gedorn-Jensen.
						Local anesthesia with 5 cc. procain.
10:20						Trepanation.
10:40						Punctured.
						No tetani in trunk & extremities.
10:50	36.7	14	0.128	16	48	Pupils constricted, pupil reflex ±, quietly
						laid down, on mechanical stimuli struggles
12:30	36.0	14	0.112	16	46	for 10 sec.
13:00	35.1	12	0.129	16	50	Transported from operation room to our exp. room. Abundant salivation.
14:00	34.8	13	0.124	12	50	Salivation still abundant.
15:00	34.2	14	0.120	14	54	Quietly lie down.
16:00	34.2	12	0.120	12	50	Salivation diminished.
17:00	33.7	11	0.106	12	48	No shiverings throughout.
18:00	33.2	9	0.099	12	48	
20:00	33.2	9	0.104	12	80	On mech. stimuli struggles for 15 sec.
21:00	33.2	9	0.101	12	82	
2. III						Blood samples from r. fem. a.
9:30	26.4	4	0.184	11	80	No. salivation, shiverings appeared, mouth mucosa apts to dry.
10:30	25.2	5	0.185	14	76	Pupils constricted, pupil reflex—
11:30	24.7	6	0.224	16	76	Same, shiverings increased.
12:30	24.2	6	0.218	22	64	"
18:30	23.9	7	0.226	24	45	"
14:30	23.7	9	0.236	24	60	Heart weak.
15:30	23.6	10	0.240	22	60	Blood clotted readily.
16:00	23.5	10	0.246	22	56	Shiverings exaggerated.
17:00	23.4	10	0.236	28	52	
18:00	23.4	10	0.240	32	60	
20:00	23.2	9	0.235	28	48	
21:00	23.0	9	0.206	28	46	
21:20	22.9	9		42	36	
21:30						Died.

The Tohoku Journal of Experimental Medicine, Vol. 53, No. 1, 1951

Studies on Cholinesterase

I. On the Distribution of Cholinesterase in the Human Brain

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(Received for publication, July 2, 1951)

Various researches on cholinesterase (ChE) have now clearly identified two sorts of cholinesterases, namely, specific or true ChE and non-specific or pseudo-ChE. The distribution of these ChEs in the body is different according to the species of the animal. In man, specific ChE is found in nervous tissue, muscle tissue and red blood cells, and non-specific ChE in serum, liver, spleen, pancreas and kidney. According to the present knowledge, specific ChE has direct relation with the metabolism of acetylcholine and the function of cholinergic nerve, while pseudo-ChE is much less important in this respect. Therefore, the research on the concentration of the specific ChE in the nervous system has another importance aside from the study of the pseudo-ChE in the serum. Nevertheless there is scarcely any report on the concentration of ChE in the human nervous system except probably the only one report by Nachmansohn[1], which showed the distribution of ChE in the human brain compared with those of animals. It seems, however, there is as yet no report on the distribution of ChE in the human brain, studying the relationship between the concentration of ChE and the sorts of disease. Believing the importance of the subject, we began the present study by using the autopsy materials of eight patients who died of various diseases. In our research, as far as the results from these eight cases are concerned, the constancy and characteristic distribution of ChE were ascertained in those human brains.

Method

The concentration of ChE was measured following the method of Ammon[2] using the Warburg's apparatus. Acetylcholine is hydrolised by ChE into acetic acid and choline, and carbon dioxide freed by acetic acid from sod. bicarbonate in Ringer solution is subsequently measured manometrically. The brain sample was taken from autopsy material as early as possible, cut in slice of about 0.6 cm. thick at the part of corpus

mamillare in the frontal direction. Grey and white matter of cerebrum and basal ganglions were cut off macroscopically. In addition, cortex of cerebellum was also taken. When it is impossible to measure immediately, the tissue was stored in a refrigerator one or two days. Although the interval of the death of the patient and the time of the measurement was not always constant, it gives practically no drawback to the measurement as the loss of the ChE in this deflected period was relatively small. In fact, we sometimes obtained even higher ChE value in the material which was stored longer than the other.

The brain tissue was homogenized in Ringer solution with 0.025 M sod. bicarbonate, using Potter-Elvehjem homogenizer. According to the varied concentrations of ChE among the parts of the brain, we diluted these tissues respectively in different ways so as to attain optimal concentration for measurement.

TABLE I

Dilution of the Tissues

Part of brain	Ncl. caud. Putamen	Cortex of cerebellum	Grey matter of cerebrum, Thalamus, Pallidum, Ncl. amygdalae	White matter of cerebrum
Final dilution of tissue with Ringer solution	500×	200×	100×	50×

As substratum, acetylcholine chloride (Ach) (Roche) was used. After equilibrium was obtained, Ach solution in the side bulb was poured into the main part and mixed with the tissue homogenate. The final concentration of Ach was 0.25%. A few minutes after mixing, reading was taken and repeated after ten and twenty minutes. The change of pressure in this period progressed in a straight line.

0.3 cc. of the homogenate of the tissue, which was diluted 20 times, was completely dried in the electric thermostat at 100° C and its weight was measured with the microchemical balance. The concentration of ChE is expressed in CO_2 cmm., which is being liberated from Ringer solution per 1 hr. per mg. dry tissue.

Results

The distributions of ChE in eight human brains are shown in Table II. The order according to the concentration is as follows:

Putamen, Ncl. caudatus, Cortex of cerebellum, Pallidum, Ncl. amygdalae, Thalamus, Grey matter of cerebrum, White matter of cerebrum.

The concentration of ChE is found varying in a wide range of from 200 to 0.5 CO_2 cmm. per hr. per mg. dry tissue.

The relative values of ChE in other parts compared with that of the grey matter of cerebrum is shown in Table III. According to this table,

1) The value for the white matter of cerebrum is beneath one fifth of that for the grey matter of cerebrum. (Average 0.13 times)
2) The value for Thalamus is about 2.2 times.
3) ,, ,, ,, Ncl. amygdalae is about 4.5 times.
4) ,, ,, ,, Pallidum is about 6.5 times.
5) ,, ,, ,, Cortex of cerebellum is about 8.6 times.
6) ,, ,, ,, Ncl. caudatus is about 29.9 times.
7) ,, ,, ,, Putamen is about 41.6 times.

TABLE II

The Distribution of ChE in the Human Brain

Figures express CO_2 cmm., per mg. dry weight of the brain tissue.

No.	1	2	3	4	5	6	7	8	Average
Sex and age	♀19	♂39	♂75	♂36	♂45	♀44	♀61	♀36	
Clinical diag.	Tbc. meningitis	Gastric cancer	Cancer of lung	Acute yellow liver atrophia	Subacute bacterial endocarditis	Miliary tbc.	Syphilis of spinal cord	Pulmonary tbc. peritonitis tbc.	
Grey matter of cerebrum	3.8	4.4	3.8	4.9			4.5	6.5	4.7
White matter of cerebrum	0.8	0.6	0.7				0.4	0.6	0.6
Ncl. caud.	71.1	174.9	95.4	181.1	188.1	111.0	172.8	132.5	140.8
Putamen	208.5	143.8	186.3	241.8	194.0	193.6	194.4	203.0	195.7
Pallidum	10.4	33.7	14.7	36.0	28.8	20.0	70.2	31.8	30.7
Ncl. amygdalae	21.6		14.0					28.4	21.3
Thalamus	7.3	12.2	15.6	8.1	14.0	4.3			10.3
Cortex of cerebellum	29.7	56.4	48.3	39.8		27.3		42.5	40.7

While the differences among the values for various parts of the brain are remarkably large, ChE values for the corresponding parts of the different brains vary relatively slightly. Accordingly it was not successfully concluded from the results of these eight cases whether the disease brings any definite change in ChE value or not.

TABLE III

The Relative Value of ChE in the Human Brain

No.	1	2	3	4	5	6	7	8	Average
Sex and age	♀19	♂39	♂75	♂36	♂45	♀44	♀61	♀36	
Clinical diag.	Tbc. meningitis	Gastric cancer	Cancer of lung	Acute yellow liver atrophia	Subacute bacterial endocarditis	Miliary tbc.	Syphilis of spinal cord	Pulmonary tbc., peritonitis tbc.	
Grey matter of cerebrum	0.8	0.9	0.8	1.0	—	—	1.0	1.4	1.0
White matter of cerebrum	0.17	0.13	0.15				0.09	0.13	0.13 (0.09–0.17)
Ncl. caud.	15.1	37.2	20.2	38.5	40.0	23.6	36.7	28.2	29.9 (15.1–40.0)
Putamen	44.4	30.6	39.6	51.4	41.2	41.1	41.4	43.2	41.6 (30.6–51.4)
Pallidum	2.2	7.2	3.1	7.7	6.1	4.3	14.9	6.8	6.5 (2.2–14.9)
Ncl. amygdalae	4.6		3.0					6.0	4.5 (3.0–6.0)
Thalamus	1.6	2.6	3.3	1.7	3.0	0.9			2.2 (0.9–3.3)
Cortex of cerebellum	6.3	12.0	10.3	8.5		5.8		9.0	8.7 (5.8–12.0)

Each figure is the ratio of respective value to the average value of grey matter of cerebrum.

Discussion

It is known that the concentration of ChE in the brain varies widely according to the portion thereof and the species of the animal. It is also known that the concentration of ChE for the corresponding part of the respective brains of the same species of the animal is very constant[8]. As these results are confirmed mainly by the researches using the brains of animals, it is very interesting to study the distribution of ChE in the human brain and the variation thereof caused by the disease of the patients. In the present study, although the number of brains measured was few, the characteristic distribution of ChE in the brain was confirmed. Though the units of ChE values are not the same between our report and that of Nachmansohn[8], both results are, when recalculated, in good accord.

So far as these eight cases are concerned, the relationship of ChE in the brain and the diseases of patients is not clear, but we expect to make further reports in this respect when more data are accumulated.

Summary

The distribution of ChE in human brains was studied with eight

autopsy materials. The concentration of ChE in the white matter of cerebrum is less than one fifth of that in the grey matter of cerebrum.

Basal ganglions and cortex of cerebellum had larger content of ChE than the grey matter of cerebrum. Putamen and Ncl. caudatus had extraordinarily high concentration of ChE.

There is some slight variation of the ChE content among the similar portions of these eight brains, but the relationship between the ChE concentration in the brain and the disease of the patients was not clearly observed owing to the small number of test cases.

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The Tohoku Journal of Experimental Medicine, Vol. 53, No. 1, 1951

Studies on Cholinesterase

II. On the Cholinesterase of the Human Serum

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(Received for publication, July 2, 1951)

In the first report (published elsewhere), we studied the distribution of the cholinesterase (ChE) in the human brain. In this report, we studied the ChE in the human serum. According to the recent literatures on ChE, the physiological role of the non-specific or pseudo- ChE in the serum is not so clear as the specific or true ChE in the nervous tissue. While the latter is one of the important substances for the normal function of the nerve, it is not determined whether the pseudo-ChE in the serum is indispensable or not for the normal metabolism of the body. Despite these facts, the presence of the definite relationship between serum ChE and liver made it clinically important to study the level of ChE in the serum. Furthermore, there may be some correlation, though indirect, between the serum ChE and the neuro-humoral function of the body. In this research, we studied the ChE level of the normal and of patients of liver, tumor, tuberculosis and other various diseases.

Method

As in the first report, the method of Ammon[1] using the Warburg's apparatus was employed. The serum was diluted 50 times with the Ringer's solution with 0.025 M sod. bicarbonate. As substratum, acetylcholine chloride (Ach) (Roche) was used. After equilibrium was attained, Ach solution in the side arm was poured into the main part, which contained the serum diluted with Ringer's solution. Final concentration of Ach was 0.25% (0.015 M). Though this concentration is not the optimal one for the maximal activity of the serum ChE, it was convenient to use this concentration for measuring the ChE of serum and nervous tissue simultaneously. A few minutes after mixing, reading was taken and repeated after 10 and 20 minutes. The activity of ChE was expressed

with CO_2 cmm., which is liberated from the Ringer's solution per minute per cc. serum.

Results and Discussion

1. *Normal persons*

In Table I, the value of the serum ChE in normal persons from September through December is expressed. There is no relationship between the serum ChE and sex, age or the slight change of serum protein. We can find also no increase or decrease of the serum ChE during this period of four months. Average value is 65.4, and the extent of the variation is from 50 to 90. The wide variation of the serum ChE among the normal persons has been shown by Hawes and Alles[2] and Bracht[3].

2. *Liver diseases*

The value of the serum ChE in the patients of the liver diseases was shown in Table II. The decreased value was obtained in almost every case. The results of several tests of the liver function are seen in the same table. Among these tests, the decrease of the serum ChE has close connection with the positive results of the bromsulfaleine test. Some of the cases which have the decreased value of the serum ChE showed the normal results in Takata-, Weltmann-, thymol-, γ-globulin- (Kunkel), hippuric acid-test, bilirubin in serum, urobilinogen in urine, and blood cell sedimentation rate. In three cases of the intoxication of para-nitrochlorbenzene, which showed normal liver function, the value of the serum ChE was also in the normal range. In three cases (No. 3, stenosis venae hepaticae, No. 5, livercirrhosis with liver cancer, and No. 7, cholelithiasis), the measurement of the serum ChE and the tests of the liver function were repeated along with the advance of the clinical feature. In the case of stenosis venae hepaticae, the patient recovered markedly in 5 months, ascites disappeared, serum protein increased from 5.4 to 7.3 g. per cent, bromsulfaleine test changed from 30 to 8. The serum ChE value increased gradually, 10.8 at first, 20.3 after 80 days, 31.9 after 100 days, and 49.8 after 160 days. In the case of livercirrhosis with liver cancer, the state of the patient became worse. The value of bromsulfaleine test changed from 15 to 35 in 75 days. The ChE value decreased from 50.7 to 37.0. In the case of cholelithiasis the state of the patient did not show the marked change. The serum ChE value was 20.2 at first and 26.9 after 80 days. According to the work of Brauer and Root[4,5], the serum ChE decreases when the liver function of the experimental animal is damaged with carbon tetrachloride, and returns to normal when the liver function recovered. In the clinical investigation, Antopol et al.,[6] McArdle[7], Hazard[8] observed the low serum ChE value in the

TABLE I

Serum ChE in Normal Persons

September				October				November				December			
Sex	Age	Serum ChE	S.P.	Sex	Age	Serum ChE	S.P.	Sex	Age	Serum ChE	S.P.	Sex	Age	Serum ChE	S.P.
♂	24	75.8	8.6	♀	53	78.4		♀	33	52.5	7.3	♂	41	54.2	7.4
♂	30	60.6	7.7	♀	28	50.0	7.4	♂	24	80.7	8.2	♂	21	60.8	8.2
♀	22	57.7	7.3	♂	24	55.9	7.7	♂	21	60.8	8.2	♂	24	49.9	
♀	25	54.5	7.6	♀	30	96.2	8.4	♂	50	61.1		♀	32	60.9	7.5
♀	24	57.4	7.5	♂	24	76.2	8.4	♀	33	52.2	7.3	♂	22	59.5	7.3
♀	24	58.0	8.3	♀	24	62.5	7.6	♀	22	70.8	8.8	♀	22	81.0	7.2
♀	20	67.0	8.6	♀	29	64.0	7.3	♀	34	69.2	8.3	♂	29	78.5	7.9
♀	27	70.0	7.3	♀	24	59.4	7.9	♀	21	89.4	8.3	♂	19	70.6	7.3
♂	30	62.6	8.1	♀	22	84.5	9.2	♀	18	64.8	8.3	♀	22	75.8	8.2
♂	24	54.5	8.3	♀	24	72.1	7.4					♂	22	73.1	7.4
Variation 55–76				Variation 59–96				Variation 52–89				Variation 50–81			
Average 61.9				Average 66.8				Average 66.8				Average 66.4			

Serum ChE is expressed in CO_2 1 min. 1 cc. serum.
S. P.=Serum Protein.

patients of the liver diseases. In our results, as shown above, the close relationship between the serum ChE and the liver is clear. But the change of the serum ChE is not correlated with the results of every liver function test. The decrease of the serum ChE do not always accompany the change of the Takata-, Wletmann-, thymol-, γ-globulin-, and hippuric acid-test. Though in most cases total serum protein and serum ChE level change simultaneously, we can find sometimes the normal or high total serum protein in the cases of the low serum ChE. As far as our results are concerned, the value of serum ChE is better correlated with results of the bromsulfaleine test than those of other liver function tests.

3. *Neoplastic diseases*

In most cases of 16 patients of neoplastic diseases the low serum ChE levels of the patients of neoplastic diseases are prevalent. Especially in the case of gastric cancer a remarkably low level is observed. It is well known that the activities of the serum esterase and lipase of the tumor-bearing animals and tumor patients are low[9,10,11,12]. Our results are also in accord with these literatures. The marked decrease of the serum ChE of the patients of gastric cancer will be explained by the most serious disturbance of the liver function of the patients of gastro-intestinal tumor compared with that of the other tumor patient[13]. But it is interesting to

TABLE II

Serum ChE in the Patients of Liver Diseases

No.	Clin. diag.	Sex, age	Serum ChE	Liver function tests									
				Takata	Weltman	Thymol	γ-gl.	Bromsulfaleine	Hip. acid	S.P.	M	U	B.S.R.
1	Livercirrhosis	♀ 36	17.0	6	6	4.0	11.0	17		6.3	3	++	86
2	"	♂ 56	25.0	6	11	6.2		20	36.2	7.3	10	+	63
3	Stenosis venae hepaticae	♂ 25	10.8	2	6	4.2	10.0	30		5.4	11	++	3
4	Gastric cancer with liver metas. and peritonitis carc.	♂ 30	37.6		9	5.0	11.0	50		7.2	25	++	18
5	Livercirrhosis with livercancer	♂ 46	43.3	6	9	7.4	21.0	15	23.0	9.6			
6	"	♂ 51	23.8	0	8	3.1	13.8	25	48.9	6.6	10	++	28
7	Cholelithiasis	♂ 50	20.2	0	9	0.6	4.6	80		7.1	171	+	28
8	Icterus catarrhalis	♀ 45	49.3			4.7	15.8	15		9.6	12	++	63
9	"	♂ 48	35.5	2	7	5.0	9.5	18	53.3	7.5	36	+	14
10	Banti's disease	♂ 54	60.1	0	8	1.8	12.3		34.9	7.2	4	+	14
11	Syphilis of spinal cord with livertumor	♂ 48	35.5	0		3.5		35	31.3	6.8	16	+	12
12	P-chlornitrobenzen intoxication	♂ 33	55.6			0.3	3.4	7	63.2	7.7	8	++	8
13	"	♂ 44	59.1	0		1.4		4	65.2	6.7	7	+	5
14	"	♂ 36	73.0	0		1.4	3.7	4	52.7	7.6	8	++	10
	Normal value		50–90	1–2	5–7	0–4.0	4–12.0	<5	>50	6.5–8.0	<5	+	<10

Hip. acid=Hippuric acid, S.P.=Serum protein, M=Meulengracht (Bilirubin in serum), U=Urobilinogen in urine, B.S.R.=Blood cell sediment. rate, γ-gl.=gamma globulin

note, that in these tumor patients, the results of the liver function test are not so bad as in the cases of the liver diseases. As described above, therefore, it is shown also in these tumor patients, that the change of the serum ChE is one feature of the liver function, which is distinguished from the results of other liver function tests.

4. *Tuberculosis*

Table III shows the serum ChE of the tuberculous patients. In some cases such as No. 9 (pulmonary tbc.), No. 15 (exsudative pleurisy), and No. 24 (tbc. meningitis), the serum ChE is very low. There are reports of the decrease of serum ChE in the serious cases of tuberculous patients[10)11)]. However, Levine and Hoyt[12)] observed no decrease of the serum ChE of the patients of pulmonary tuberculosis. According to our study, the general change of the serum ChE in tuberculosis is not always in the direction of decreasing, and thus differed from the type of change in the cases of liver and neoplastic disease. Certain patients showed the higher value of the serum ChE than that of the normal and the variation of the serum ChE level of the tuberculous patients is in a very wide range.

TABLE III

Serum ChE Values in Tuberculous Patients

No.	Clin. diag	Sex, age	Serum ChE	Serum protein	Fever	B.S.R.	Tbc.* bac.	Roentgenograms etc.
1	Pulm. tbc.	♂23	56.0	6.5	normal	3	VIII	
2	"	♂26	97.7	8.3	"	33	VI	
3	"	♂31	40.9	6.5	slight	33	III	
4	"	♀22	90.6	8,3	"	58	0	
5	"	♂17	33.6	7.3	slighter remittend	47	VII	
6	"	♂43	92.8	8.9	normal	13	0	Shadows with or without cavities
7	"	♂32	62.4	7.3	slight	27	II	
8	"	♀21	83.5	7.9	"	45	VI	
9	"	♀25	15.4	7.5	"	58	VII	
10	" with right exud. pleuritis	♂23	53.1	7.4	"	83	II	
11	" and exud. pleuritis	♂31	58.1	7.9	normal	34	I	
12	Mil. tbc.	♀18	42.6	8.0	slight	62	III	
13	Exud. pleuritis	♂24	77.0	7.7	normal	3		
14	"	♂22	76.6	7.4	"	7		Pleural effusion
15	"	♂44	16.9	7.5	high	40		
16	"	♂45	42.3	6.8	slight	83	0	
17	Adh. pleuritis	♀22	78.2	7.3	"	4		
18	Tbc. peritonitis	♀18	84.1		"			
19	"	♀35	46.8	7.6	normal	51		
20	"	♀41	67.9	7.1	slight	6		
21	"	♀24	51.2	7.1	normal	11		Normal lung
22	" and intest. tbc.	♀34	39.1		slight	15		
23	Spinal caries	♂25	66.3	7.0	"	35		
24	Tbc. meningitis	♂36	17.5	7.2		46		

Tbc. bacilli in direct sputum smear is expressed in Gaffky scale.

5. *Disease due to endocrine disturbance*

The serum ChE of 17 patients of the internal secretion were studied. All values are in the range of those for normal persons. The relationship between serum ChE level and the function of endocrine glands, if there exists any, becomes important, because the participation of serum ChE in the neurohumoral state of the body may be proved by this probable relationship. Some workers reported that the serum ChE was high in hyperthyroidism and decreased with adequate antithyroid therapy[14)15)16)]. In contrast to these clinical observations, it is reported experimentally that the pseudo-ChE of the serum in rats increases after thyroidectomy[17)].

We could not find out the increase of the serum ChE in Basedow's disease. One explanation is that, though the hyperchyrodism may have influence on the serum ChE level, the concurrent liver damage may cause the decrease of it. Most of the disturbance in the patients of the internal secretion has the pluriglandular nature. Thus, the same consideration as in hyperthyroidism will be necessary for the serum ChE level in the endocrine diseases. The repeated measurements of the serum ChE level for one patient in the progress of the disease will be of use for obtaining the exact results by minimizing the influence by the liver. Untill such data are obtained, we shall refrain from further discussion.

6. *Nervous disease*

12 cases were studied. All values are in the range of the normal ones. The relationship between the serum ChE level and the nervous diseases, especially myasthenia gravis, was studied by many workers, however, without any decisive conclusion[18]. Furthermore, as described in the preface, it was demonstrated in the biochemical research that the role of the serum ChE in the acetylcholine metabolism is much less important than the true ChE present in the nervous or muscle tissue. According to our results, no relation was found between the serum ChE and any of the nervous diseases.

7. *Various diseases*

In the serious cases such as erythematodes, subacute bacterial endocarditis, the serum ChE levels are very low. In other diseases, however, no characteristic change of the serum ChE was found. It was reported in the literature that the serum ChE decreased in malnutrition and returned to normal in recovery[19]. The decrease of serum ChE in the serious state of many diseases may be due to the concurrent malnutrition or liver disturbance.

8. *ChE value in the red blood cells*

In Table IV, the ChE values of the red blood cells and of the serum in 1 cc. of the whole blood are shown. The whole blood added with citrate is centrifuged and the hematocrit is measured. The red blood cells were washed twice with the saline and hemolysed by adding the distilled water to the same volume as the original whole blood. The ChE value of red blood cells was expressed in terms of the CO_2 cmm. per 1 minute per red blood cells in 1 cc. whole blood. The ChE value of the serum was expressed with CO_2 cmm. per 1 minute per serum in 1 cc. whole blood, or CO_2 cmm. per 1 min. 1 cc. serum $\times \left(1-\frac{\text{Hematocrit value}}{100}\right)$. As shown in Table IV, the ChE value of red blood cells was much higher than the

TABLE IV

ChE in Human Red Cells and Serum

No.	Clin. diag.	Red cell* ChE	Serum† ChE	Hematocrit%	Hemoglobin %
1	Stenosis venae hepaticae	104.1	4.8	56	120
2	Progressive muscular dystrophy	94.9	26.1	42	131
3	Livercirrhosis with livercancer	92.5	20.8	52	116
4	Spontaneous gangrene	79.5	33.8	40	70
5	Gastric cancer	72.5	12.8	36	85
6	"	66.5	11.4	22	45
7	Hypophyseal cachexia	65.5	27.1	28	86
8	Basedow's disease	59.5	35.5	29	75
9	Progressive paralysis	56.8	29.5	32	112
10	Ankylostomiasis	55.9	38.8	22	41
11	Anemia (sepsis ?)	2.9	36.6	5	25

* Red cell ChE is expressed in CO_2 cmm./min., red blood cells in 1 cc. whole blood.

† Serum ChE is expressed in CO_2 cmm./min., 1 cc. serum $\times (1-\frac{\text{Hematocrit value}}{100})$

serum ChE. The former correlates fairly well with the value of hematocrit and hemoglobin. The serum ChE has no relationship with them.

SUMMARY

The serum ChE level in the normal persons and the patients of various disease was determined manometrically. The value was expressed in terms of CO_2 cmm. per 1 min. per 1 cc. serum freed from the Ringer's solution of 0.015 M acetylcholine chloride (Roche).

1) Average value of serum ChE in normal persons is 65.4. The variation is from 50 to 90.

2) The serum ChE level in the patients of liver- and neoplastic diseases is low. The decrease of the value is remarkable in the cases of serious damage of the liver and in gastric cancer. The decrease of serum ChE can occur despite the negative results of many liver function tests. The serum ChE level correlates with the results of bromsulfaleine test better than those of other liver function test.

3) In pulmonary tuberculosis, the variation of the serum ChE level is in a very wide range. Serious cases showed very low value, while in others normal or higher level was found.

4) In the cases of the endocrine disturbance, nervous disease or other various diseases, no characteristic change of the serum ChE level was found. However, regardless of the sort of disease, the serum ChE level is low in the serious cases.

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The Tohoku Journal of Experimental Medicine, Vol. 55, No. 1, 1951

An Experimental Study of Cerebral Blood Flow in Hypertension

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(Received for publication, July 9, 1951)

In hypertension, disturbance in cerebral blood flow which used to occur in this disease can be considered one of the most important factors for its clinical symptoms, moreover some presumption can be expected also of the genesis of cerebral hemorrhage by investigating the disturbed brain circulation; namely a lot of cerebral symptoms, such as insomnia, headache, tinnitus, vertigo, reduced power of concentration, failing in memory, mental irritability, etc. are attributed usually to the congestion or deficiency of oxygen supply in brain. It has been pointed out by a series of investigators such as Roy and Sherrington,[1] Kawaguchi[2] Yamakita and others, that in case of raised blood pressure the blood flow in the brain undergoes to some alternation, while Williams[3] and Kety and coworkers[4] will deny this fact.

In order to give some contribution to this problem which is not yet settled, the present experimental investigation has been undertaken, measuring the cerebral blood flow of the rabbits in the earlier and later stage of hypertension which was induced in them experimentally by the method of Goldblatt.[5]

Experimental

Method

Rabbits weighing about 2.5 kg. were employed, those which show extraordinarily variable blood pressure levels in healthy condition being excluded. In them the renal ischemia of desired degree was induced by means of Goldblatt's method, a specially devised artery silver clamp, with a graduated acting screw and compressing plate being applied to the main renal artery, so that the grade of arterial constriction could be varied and controlled to the desired degree. The blood pressure was measured in ear of rabbit frequently for a long while during the course of experiments

by using conservative method of Kuraya.[6] This original method was modified, the central auricle artery in its most dilated condition being brought at a definite point peripheral of the tambour and the pressure acted upon when the blood flow has begun again to pass through that point with pulsation being read; the readings thus obtained were nearly the same with those obtained by the method of Kawaguchi.[7] The fluctuation of blood pressure could be also measured sensitively.

There are devised various methods for the determination of cerebral blood flow in animals. Yamakita,[8] perceiving that the superficial temporal vein in rabbit communicates with transversal sinus through temporal emissarium and pours its venous blood into the external jugular vein, determined the cerebral blood flow by measuring the outflow of venous blood from the cannula inserted in the superficial temporal vein after tying the other branches which do not communicate to the temporal emissarium. In the present experiments Yamakita's method was employed, thereby also the oxygen content of the blood samples thus obtained from the superficial temporal vein was compared with that of arterial blood from femoral artery. For the determination of the oxygen content of the blood Barcroft's differential blood gas apparatus was used. The values obtained by Fleischl-Miescher's hemoglobinometer were applied as the criteria determining the blood concentration.

In a series of experiments the carotid gland and neighbouring nervous plexus around carotid sinus in an extent of 10 mm. on both sides were extirpated, in order to observe the influence of the carotid sinus upon the cerebral blood flow in hypertensive animals. In a small group of rabbits with hypertension the findings of ocular fundus were observed by means of electrical ophthalmoscope.

Results

I. Control experiments on normal rabbits (Table I).

TABLE I

Mean Values in Normal and Hypertensive Rabbits

	Blood pressure mm Hg	Oxygen content Vol. %		Oxygen consumption O_2	Oxygen consumption rate %	Venous blood flow (v) c.c.	$\Delta O_2/V$	ΔO_2V
		Arterial	Venous					
Normal	76.2	18.05	11.50	6.55 (1.27)	36.4 (7.16)	2.5 (0.73)	2.94	16.51
Hypertensive	106.0	18.03	13.40	4.63 (1.97)	25.7 (8.08)	2.91 (1.31)	1.96	13.47
Changes in %	+39.6	0	+16.50	−29.3	−32.9	+16.4	−33.3	−19.8

The figures in parenthesis express unbiased disperse.

Of 17 rabbits, 9 were experimented on one side of the superficial temporal vein and 8 rabbits on its both sides at interval varying 2 to 5 days. The measurements yielded following data: The mean value of the oxygen consumption (ΔO_2) is 6.55 (vol %), its unbiased disperse 1.27. The oxygen consumption rate ($\Delta O_2/AO_2$) on an average 36.4%, its unbiased disperse 7.16. The amount of the venous blood flow from the superficial temporal vein per minute (v) is in the mean 2.5 cc,, its unbiased disperse being 0.73. The quotient of oxygen consumption by the venous blood flow is on an average 2.94. The mean value of the product of oxygen consumption and the venous blood flow 16.51.

II. Experiment on hypertensive rabbits. (Table I)

In 16 rabbits the artery clamp was applied to the main renal artery on one or both sides in order to induce arterial hypertension by renal ischemia. In the first series of the experiment (9 rabbits), in which the changes in the earlier stage of hypertension were to observe, the artery clamp yielding about 1 mm. calibre to the renal artery was applied only on one side. In the majority of the cases apparent rise in the blood pressure appeared in one or two days after operation and continued for about one week, afterward used to discontinue. In one case its duration was longer than 10 days. In another one case the animal died shortly after the rise of blood pressure and in 2 cases there could be no significant change in blood pressure observed.

The ischemic kidney showed atrophy or cloud swelling and in the majority ischemic infarcts of irregular form in the cortical area where microscopically severe parenchymatous degeneration or necrosis could be seen. No change in the control kidney. Weight of kidneys on an average: 9.1 g. on ischemic side, 10.7 g. on control side.

In the second series of the experiments which numbered 7, the changes in the later stage of hypertension were investigated. The experimental constriction of the main renal artery was carried out on both sides, one after another in two sittings with the interval varying from 1 to 7 days. On the first side the calibre of the artery was allowed about $\frac{3}{4}$ mm., on the second side about $\frac{1}{2}$ mm.

The elevation of the blood pressure occurred in one or two days after the first constriction and continued in general more than 3 weeks, though in a few cases it showed temporarily a slight fall immediately after the second arterial constriction on the other side. In 2 cases the animal died in 2 weeks and in 3 within a few days after the first constriction with sudden rise of blood pressure. In on case the blood pressure remained in normal level..

In kidneys of those animals with 2 exceptions the atrophy was more significant on the side in which the clamp had been applied at first than

on the another side. In 2 cases, in which the increased blood pressure maintained the high level for more than 25 days, the kidney of the first side showed resp. 3.2 g. and 2.5 g. in weight and the parenchyma was almost replaced by connective tissues. The mean weight of the kidneys with the first cramping was 10.06 g. and that of those with the second cramping 11.5 g.

The data obtained by the measurement fluctuated in a wide range as it was in control experiments. The rise of blood pressure after constriction of the main renal artery was on an average 39.6%, the mean increase of the pulse rate was 6.1%, that of respiration rate 16.1%. Those changes in the pulse and respiration rate were significant in the initial stage of hypertension and lessend later.

Oxygen consumption (ΔO_2) in the brain and its rate ($\Delta O_2/AO_2$):

The difference between the arterial and venous oxygen contents, that is, the oxygen consumption in the brain was 4.63 (vol %) on an average. This value is 29.3% less compared with that in control experiments. Its unbiased disperse is 1.97. The calculated oxygen consumption rate is 25.7, thus being 32.9% less than that in control experiments; the unbiased disperse is 8.08.

The venous blood flow from superficial temporal vein:

This was in the mean 2.91 c.c. per minute, which is 16.4% more than that in control experiments; the unbiased disperse is 1.32.

Quotient of oxygen consumption by venous blood flow:

1.96 on an average, which is 33.3% less than that in control experiments.

Product of oxygen consumption and venous blood flow:

The average value is 13.47 and 19.8% less than that in control experiments.

The changes above mentioned were more significant in the initial stage (within 3 days after the arterial constriction) than those in the later stage of hypertension (Table II). The same is also true in those experiments in which the renal ischemia was caused on one and the same rabbit twice one side after another in a certain interval (Figg. 1 and 2), the difference of the data between two stages being much greater, compared with those obtained in two similar stages in one and the same normal rabbit.

The difference of blood concentration between the venous blood flowing out from the brain and the arterial blood:

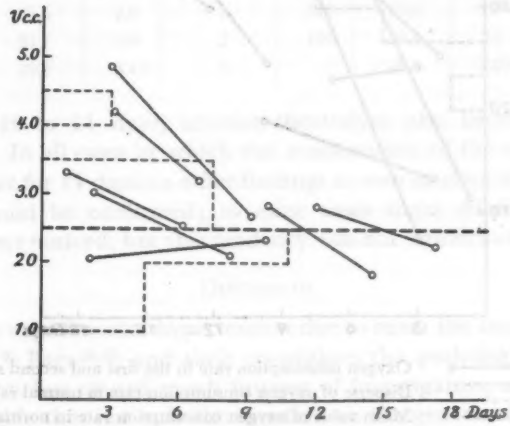
In normal rabbits, the difference of blood concentration observed by the hemoglobin value between the superficial temporal vein and femoral artery is very slight. The mean value of 5 cases is 0.2%, while this value amounts in rabbits with hypertension to 5.9% on an average of 20 experiments. This tendency is more apparent in the early stage of hypertension, namely 7.63%, and lessens later, the mean value being reduced to 3.74%.

TABLE II

Mean Values Obtained in 2 Stages of Hypertension

	Blood pressure mm Hg	Oxygen content Vol %		Oxygen consumption ΔO_2	Oxygen consumption rate %	Venous blood flow (v) c.c.	$\Delta O_2/V$	ΔO_2V
		Arterial	Venous					
Earlier stage	102	17.96	13.96	4.00	22.3	3.3	1.36	13.20
Later stage	108	18.28	12.79	5.49	30.1	2.6	2.37	14.22

Fig. 1. Cerebral blood flow in two stages of hypertention.



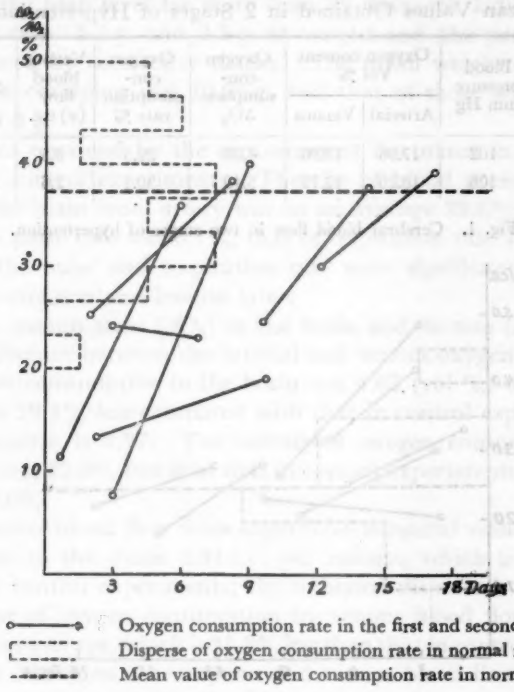
The results may be summarized as follows:

1. The oxygen consumption and oxygen consumption rate in the brain diminish remarkably after renal ischemia, but later recover nearly to the normal level.
2. The venous blood flow from the brain increases apparently after renal ischemia, diminishes later approaching to the normal value.
3. Accompanied by those changes, $\Delta O_2/V$ and ΔO_2V within three days after onset of hypertension are significantly less than those in normal animals, afterwards increasing and approaching to the normal level.

The influence of extirpation of carotid sinus upon the changes of cerebral blood flow in hypertensive rabbits

In order to observe whether carotid sinus acts or not any influence

Fig. 2. Oxygen consumption rate in the brain in two stages of hypertention.



upon the changes of cerebral blood flow of hypertensive animals, in 4 rabbits the extirpation of carotid sinus on both sides were performed. The extirpation was carried out in the later stage of the hypertension when the changes of cerebral blood flow should be minimized and after short interval the cerebral blood flow was measured (Table III). After the extirpation the blood pressure used to rise temporarily moreover. In all those animals definite changes could be seen: namely, decrease in oxygen consumption and oxygen consumption rate, increase in blood flow became remarkable again. Those changes are nearly similar to the values observed in early phase of the hypertension.

Findings in ocular fundus

In 7 rabbits with exeperimental hypertension caused by Goldblatt's method, findings in ocular fundus before and after elevation of blood pressure were examined. With sudden rise of blood pressures tortuosity and fullness of central arteries of retina could be noticed almost in every case; in 3 cases they were particularly significant. These findings dimi-

TABLE III

Values Obtained in Animals whose Carotid Sinus on Both Sides has been Extirpated

No. of exp.	Experiment before extirpation				Eqperiment after extirpation			
	Blood pressure mm Hg	Oxygen consumption rate %	Venous blood flow c.c.	Time elapsed since onset of hypertension (days)	Blood pressure mm Hg	Oxygen consumption rate %	Venous blood flow c.c.	Time elapsed since onset of hypertension (days)
24	120	31.2	3.2	2	130	33.0	4.0	16
26	100	29.2	3.5	2	130	24.0	3.0	34
27	100	25.5	2.8	2	110	29.4	2.5	17
28	90	31.1	2.9	2	105	29.4	2.8	17
Mean		29.2	3.1	5		28.9	2.07	21

nished after 10 to 11 days, arteries themselves also became narrower successively. In all cases in which the examination of the ocular fundus was carried out for 14 days no other findings as seen in clinical cases of hypertension could be confirmed; in some cases slight dilation of central retinal vein was noticed, but this tendency was not significant in general.

DISCUSSION

Since the experimental hypertension due to renin has been ascertained by Goldblatt,[9] Page[10-18] and their co-workers the pathologic physiology of hypertension has caused much interest of investigators, and it seemed once presumable that not only the question on the genesis of renal hypertension but also that of essential hypertension could easily be solved. But this is in fact not the case, the problem of hypertension remaining yet entirely undermined, though it is out of doubt that the acting point of the pressor substance lies in the wall of small vessels or capillaries which undergoes to contraction or increase of tension.

The present experiment performed on rabbits with Goldblatt's hypertension has revealed that the blood flow in the superficial temporal vein seems apparently, though not considerably, is augmented, a fact which indicates accordingly some increase of blood flow in the brain.

Arguments have been repeated on the reaction of cerebral vessels to adrenalin; the majority of investigators, even those who assert contraction of cerebral vessels by this drug accept at least transient increase of cerebral blood flow with onset of general arterial hypertension by adrenalin.[14-17] In rabbits with Goldblatt's hypertension also the small blood vessels in brain which are provided with only scanty smooth muscles and vasoconstrictor nerves, will dilate to some extent passively with sudden increase of arterial blood pressure. But its duration is too long compared

with the case of adrenalin hypertension; there should be necessarily performed more advanced researches on other agents which could create disorder on blood circulation in the brain.

On the other hand, the oxygen consumption in the brain of hypertensive rabbits diminishes remarkably even if the increase of hemoglobin concentration (or decrease of blood water) in a unit volume of the blood samples be taken into consideration. Naturally, the amount of gaseous interchange between blood and tissues depends on the extent of area of capillaries, namely their surface, where it takes place, and on the oxygen demand of tissues; especially it is modified by the velocity of blood flow in capillaries. When the blood flow in capillary increases while the oxygen consumption of the tissues remains unchanged, the used oxygen amount in a unit volume of blood should be lessened consequently. In those cases, the fact that even ΔO_2V, that is the oxygen quantity transfered into the brain tissue in every minute is decreased, indicates that owing to the increased cerebral circulation there is not time enough for the interchange of gases between blood and brain tissues. The characteristic structure of brain capillaries, as confirmed by Sepp[18] may be turned to account for the explanation of the fact above mentioned, as good as for the augmented concentration of venous blood flowing out from the brain; with onset of hypertension, the precappillaries and the postcapillaries may be dilated passively, while the capillaries in the narrow sense will remain without remarkable change in their calibre. It is readily presumed that through the dilated precapillary the blood water goes out in an increased amount into the tissue, while the postcapillaries which are also dilated, cannot take up enough tissue fluid into the blood stream. This may have some connection with the increased amount of cerebrospinal fluid in experimental hypertension, a fact recognized by Kawaguchi.[2] As a result of the dilatation of pre- and postcapillary area, the velocity of blood flow in the capillaries in the narrow sense will be accelerated and the blood pressure in them should be relatively decreased in accordance with the hydrodynamic law of continuity, thus leading to diminished oxygen discharge.

In the present experiment it has been pointed out that the above mentioned changes in cerebral blood flow are more significant in the early stage of the experimental hypertension, and minimized gradually in the later stage. And as this latter phenomenon can occur without any lowering of the blood pressure, it may be presumed that some new changes must take place in the cerebral blood flow. It is generally acknowledged by excellent works of Hering[19] and Heymans,[20] that the blood flow in brain is controlled by carotid sinus. Our experiment revealed that after denervation of the carotid loop the changes in cerebral blood flow became significant again instead of approaching to normal level. Indeed, there

may happen in the course of time contraction of cerebral arterioles overcoming the increase of blood pressure; but those facts above stated support the presumption that the carotid sinus displays an action though scanty, gradually to bring the cerebral blood flow in normal condition.

Unevenness and tortuosity of retinal artery which were observed frequently in present investigation in the course of elevation of the blood pressure, have been pointed out also by Goldblatt,[21] Kinukawa,[22] Kuwajima[23] and others.[24] Those findings appeared in accordance with the change in the cerebral blood flow, which are regulated step by step by carotid sinus or by arterioles themselves.

Thus, it is ascertained that there are at least two phases in the change of cerebral blood flow in arterial hypertension, and it can be easily understood that there exist certain differences between experimental hypertension induced rapidly and the clinical hypertension, which comes about gradually under the compensatory action of blood pressure regulating system including carotid sinus.

The experimental data obtained in the present investigation cannot simply be applied for understanding the pathologic physiology of the arterial hypertension in human body, where the regulation of blood supply to the brain by carotid sinus and others is much predominant in comparison with that of rabbits. Nevertheless, considering that in some patients with hypertension, the oxygen consumption in the blood circulating through the brain is lowered slightly as has been pointed out by Williams, it cannot be neglected the possibility, that there may occur similar procedure in human body too, especially in cases of hypertension with certain disturbance in blood pressure regulating system such as carotid sinus function, as was proved in arteriosclerosis, when sudden onset of high blood pressure attacks the circulation in the brain.

Unexpected seizures of cerebral hemorrhage accompanied by sudden rise of blood pressure in cases where apparent arterial hypertension could not be proved in the clinical history, which has been reported by Michisuga[25] and many other investigators, should be of interest when we consider its mechanism basing on the presumption deduced from the experimental data as above stated; rapid onset of hypertension followed by dilatation of cerebral blood vessels and increase of blood flow in the brain may bring about cerebral hemorrhage in some cases.

Conclusions

1. In rabbits with experimental hypertension caused by Goldblatt's method, especially in case of its rapid onset, the cerebral blood flow is increased and the oxygen consumption in the brain is lowered; judging by the hemoglobin content, the venous blood flowing out of the brain is con-

centrated to some extent. It may be presumed that those changes may depend partly upon the dilatation in the pre- and postcapillaries, what Sepp calls. The findings in optic fundi support this presumption.

2. These changes are more significant in the early stage of experimental hypertension and gradually minimized in the course of time. Control by carotid sinus in this procedure seems significant.

3. These findings may suggest the changes in cerebral blood flow in arterial hypertension rapidly occuring in human body, as well as the mechanism of cerebral hemorrhage in some clinical cases.

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